

07/782 255
Att#F

WEST Search History

DATE: Sunday, November 03, 2002

<u>Set Name</u> side by side	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u> result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
L13	19 with L12	77	L13
L12	activity or efficiency	1713966	L12
L11	19 with L10	0	L11
L10	loss near3 activity	15686	L10
L9	14 near5 l5	2345	L9
L8	14 with L7	28	L8
L7	(improved or increased) efficiency	51852	L7
L6	14 with L5 with l2	23	L6
L5	synthetic	692531	L5
L4	promoter	94738	L4
L3	l1 with L2	2	L3
L2	overlap\$	478532	L2
L1	synthetic promoter	554	L1

END OF SEARCH HISTORY

[Generate Collection](#)[Print](#)**Search Results - Record(s) 1 through 28 of 28 returned.**

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- ☐ 1. [20010007661](#). 08 Feb 00. 12 Jul 01. Composition containing Alpha-fodrin or Alpha-fodrin fragment protein. Hayashi, Yoshio, et al. 424/185.1; 514/13 A61K039/00.
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- ☐ 2. [6284956](#). 25 Jun 99; 04 Sep 01. Plant selectable marker and plant transformation method. Rodriguez; Raymond L, et al. 800/320.2; 435/194 435/204 435/209 435/320.1 435/418 435/419 435/468 435/470 435/69.1 800/278 800/287 800/293. C12N015/29 C12N015/82 C12N015/54 C12N015/56 A01H005/00.
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- ☐ 3. [6284252](#). 12 Jun 98; 04 Sep 01. Transdominant TAT variants of the human immunodeficiency virus. Mehtali; Majid, et al. 424/208.1; 424/188.1 435/69.1 530/300. A61K039/21.
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- ☐ 4. [6258561](#). 20 Oct 99; 10 Jul 01. Method of producing a 19P2 ligand. Masato; Suenaga, et al. 435/69.4; 435/252.3 435/320.1 435/69.1 530/350. C12N015/09 C12N001/20 C12N015/00 C12P021/06 C07K001/00.
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- ☐ 5. [6121057](#). 13 May 98; 19 Sep 00. Methods of detecting antibodies to .alpha.-Fodrin and fragments thereof in diagnosing sjogrens'. Hayashi; Yoshio, et al. 436/536; 435/194 435/7.8 530/350. G01N033/536.
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- ☐ 6. [6103882](#). 26 Jun 98; 15 Aug 00. Method of producing a 19P2 ligand. Masato; Suenaga, et al. 530/407; 530/324 530/399. A61K038/01 A61K038/24 A61K038/00.
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- ☐ 7. [6056967](#). 06 Jul 98; 02 May 00. Method of producing water-soluble condensates and addition products containing amino groups, and use of said condensates and addition products. Steuerle; Ulrich, et al. 424/401; 162/100 424/70.1 424/70.11 528/480 528/502A. A61K007/00 A61K007/06 C08F006/00.
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- ☐ 8. [5889175](#). 04 Jan 94; 30 Mar 99. Nucleic acids encoding HIV-1 trans-dominant mutants and their use to abrogate HIV-1 viral replication. Mehtali; Majid, et al. 536/23.72; 424/188.1 424/208.1 435/69.1 530/300 530/324 530/333. C07H021/04 A61K039/21 A61K038/00.
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- ☐ 9. [5888729](#). 07 Jun 95; 30 Mar 99. Oligonucleotide probes and methods for detecting Streptococcus pneumoniae. Kacian; Daniel L., et al. 435/6; 435/91.2 435/91.21 435/91.5 435/91.51 536/24.1 536/24.33. C12Q001/68 C12P019/34 C07H021/04.
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- ☐ 10. [5861314](#). 06 Jun 95; 19 Jan 99. Adeno-associated viral (AAV) liposomes and methods related thereto. Philip; Ramila, et al. 435/372.3; 435/458. C12N005/10.
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- ☐ 11. [5861171](#). 02 Jun 95; 19 Jan 99. Adeno-associated viral (AAV) liposomes and methods related thereto. Philip; Ramila, et al. 424/450; 424/93.2 424/93.6 435/458. A61K009/127 A61K048/001.
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- ☐ 12. [5834441](#). 12 Sep 94; 10 Nov 98. Adeno-associated viral (AAV) liposomes and methods related thereto. Philip; Ramila, et al. 514/44; 424/450 424/93.21 435/320.1 435/325 435/458 435/69.1 536/24.1. A01N063/00 A61K048/00 A61K009/127 C12N005/00.
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- ☐ 13. 5776234. 12 Aug 96; 07 Jul 98. Anionic bituminous emulsions with improved adhesion. Schilling; Peter. 516/47; 106/278 106/284.06 516/DIG.6 524/60 524/61. C08L095/00.
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- ☐ 14. 5772749. 15 Sep 97; 30 Jun 98. Anionic bituminous emulsions with improved adhesion. Schilling; Peter, et al. 106/277; 106/284.4 524/61. C08L095/00.
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- ☐ 15. 5693508. 08 Nov 94; 02 Dec 97. Retroviral expression vectors containing MoMLV/CMV-IE/HIV-TAR chimeric long terminal repeats. Chang; Lung-Ji. 435/6; 435/320.1 435/456 435/465 435/69.1 536/24.1. C12N015/00 C12N015/09 C12N015/63 C12P021/06.
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- ☐ 16. 5670562. 15 Oct 96; 23 Sep 97. Adhesion enhancers for anionic bituminous emulsions. Schilling; Peter. 524/61; 106/277 524/59 524/60. C08L095/00.
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- ☐ 17. 5668197. 12 Aug 96; 16 Sep 97. Anionic bituminous emulsions. Schilling; Peter. 524/61; 524/59 524/60. C08L095/00.
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- ☒ 18. 5667986. 01 Jul 94; 16 Sep 97. Yeast promoter for expressing heterologous polypeptides. Goodey; Andrew R., et al. 435/69.1; 435/254.2 435/320.1 536/24.1. C12P021/06 C12N001/19 C12N015/11 C12N015/63.
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- ☐ 19. 5667578. 24 Sep 96; 16 Sep 97. Adhesion promoters for anionic bituminous emulsions. Schilling; Peter. 106/277; C09D195/00.
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- ☒ 20. 5554516. 02 Dec 93; 10 Sep 96. Nucleic acid sequence amplification method, composition and kit. Kacian; Daniel L., et al. 435/91.21; 435/6 435/91.51 435/91.53 536/24.32 536/24.33. C12P019/34 C12Q001/68 C07H021/04.
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- ☐ 21. 5316558. 19 Aug 92; 31 May 94. Catalytic clean-combustion-promoter compositions for liquid hydrocarbon fuels used in internal combustion engines. Gonzalez; Frank. 44/340; 44/414 44/426 44/438 44/439 44/446 44/451. C10L001/22.
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- ☐ 22. 5009853. 18 Apr 90; 23 Apr 91. Fluid catalytic cracking regeneration with reduction of nitrogen oxide. Kovacs; Richard C., et al. 422/144; 422/145 422/147. F27B015/12 F27B015/08.
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- ☐ 23. 5006495. 26 Feb 90; 09 Apr 91. Fluid catalytic cracking regeneration. Pappal; David A., et al. 502/42; 208/113 208/164 422/144 422/145 422/147 502/43 502/515. B01J029/38 B01J021/20 C10G011/18 F27B001/20.
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- ☐ 24. 4843051. 09 Jul 87; 27 Jun 89. Fluid catalytic cracking regeneration with reduction of nitrogen emissions. Kovacs; Richard C., et al. 502/42; 208/164 422/144 422/145 422/147 502/43. B01J029/38 B01J021/20 C10G011/18 F27B001/20.
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- ☐ 25. 4192889. 08 Jan 79; 11 Mar 80. Ruminant feeds containing trichloroethyl esters of essential amino acids. Hauck; Frederic P., et al. 514/538; A61K031/24.
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- ☐ 26. 4172148. 19 May 78; 23 Oct 79. Ruminant feeds containing trichloroethyl esters of essential amino acids. Hauck; Frederic P., et al. 514/550; 514/551. A61K031/22.
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- ☐ 27. DE 69614510 E WO 9622393 A1 AU 9644992 A EP 805877 A1 JP 11503001 W AU 705802

B US 6027930 A EP 805877 B1. Bacteriophage with improved helper efficiency in gene selection - retains gene III promoter whilst gene III encoding sequence is deleted. BORREBAECK, C A K, et al. C12G001/68 C12G001/70 C12N007/00 C12N007/01 C12N015/09 C12N015/11 C12N015/62 C12Q001/70 G01N033/53 G01N033/569.

☐ 28. DD 125278 A HU 17765 T. Antioxidant and detergent additives for lubricating oils - made by reaction of alkyl phenol sulphide, alkaline earth and carbon dioxide in presence of promoter. C07C149/30 C10M001/42.

[Generate Collection](#)[Print](#)

Terms	Documents
14 with L7	28

[Previous Page](#)[Next Page](#)

[Generate Collection](#)[Print](#)**Search Results - Record(s) 1 through 50 of 77 returned.**

-
- ☐ 1. [20020160397](#). 16 Jan 02. 31 Oct 02. Oncoprotein protein kinase. Karin, Michael, et al. 435/6; 435/15 C12Q001/68 C12Q001/48.
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- ☐ 2. [20020159958](#). 16 Oct 01. 31 Oct 02. Methods for producing immunoglobulins containing protection proteins in plants and their use. Hiatt, Andrew C., et al. 424/50; 424/165.1 530/388.4 A61K039/40 A61K007/28.
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- ☐ 3. [20020142468](#). 29 Nov 00. 03 Oct 02. Methods for altering the expression of hyphal-specific genes. Sundstrom, Paula. 435/484; 435/254.1 435/254.11 C12N015/74 C12N001/16.
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- ☐ 4. [20020127570](#). 27 Sep 01. 12 Sep 02. Methods for using mutant RNA polymerases with reduced discrimination between non-canonical and canonical nucleoside triphosphates. Sousa, Rui, et al. 435/6; 435/91.2 C12Q001/68 C12P019/34.
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- ☐ 5. [20020081719](#). 14 Mar 01. 27 Jun 02. Inflammation inducible hybrid promoters, vectors comprising them and uses thereof. Massaad, Charbel, et al. 435/320.1; 536/23.5 C12N015/00 C07H021/04.
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- ☐ 6. [20020019050](#). 05 Apr 01. 14 Feb 02. Compositions and methods for helper-free production of recombinant adeno-associated viruses. Gao, Guangping, et al. 435/456; 435/235.1 C12N015/861 C12N007/01.
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- ☐ 7. [20020016980](#). 10 May 01. 07 Feb 02. Transgenic plants incorporating traits of zosteria marina. Alberte, Randall S., et al. 800/289; 536/23.6 800/278 C12N015/82 C12N015/29 A01H005/00.
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- ☐ 8. [20010049097](#). 21 Jun 99. 06 Dec 01. METHOD OF IMPROVED TRANSCRIPT EXTENSION OF NONCANONICAL TRANSCRIPTS USING MUTANT RNA POLYMERASES. SOUSA, RUI. 435/6; 435/91.5 536/23.1 536/24.1 536/25.3 C12Q001/68 C07H021/02 C07H021/04 C07H021/00 C12P019/34.
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- ☐ 9. [20010047092](#). 19 Jan 01. 29 Nov 01. Novel plant promoters and methods of use. Bruce, Wesley B., et al. 536/24.1; 536/23.1 800/278 800/295 C07H021/04 A01H001/00 C12N015/82 C12N015/87.
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- ☐ 10. [20010014335](#). 05 Apr 99. 16 Aug 01. NOVEL FUSED PROTEIN, GENE THEREFOR, RECOMBINANT VECTOR, RECOMBINANT VIRUS, AND ITS USE. SAITOH, SHUJI, et al. 424/199.1; A61K039/12.
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- ☐ 11. [6461606](#). 23 Apr 99; 08 Oct 02. Materials and methods for gene therapy. Flotte; Terence R., et al. 424/93.2; 424/93.21 424/93.6 435/320.1 435/325 435/455 435/69.1 514/44 536/23.1 536/23.5. A01N063/00 A61K031/70 C12N005/00 C12N015/00 C07H021/04.
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- ☐ 12. [6441020](#). 28 Sep 98; 27 Aug 02. Protein kinase C modulators. W.. Quick; James, et al. 514/411; 514/185 514/63 514/81 540/452 540/460. A61K031/407 C07D487/06 C07D487/08.
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- ☒ 13. 6417429. 25 Nov 98; 09 Jul 02. Transgenic plants expressing assembled secretory antibodies. Hein; Mich B., et al. 800/288; 435/419 435/468 536/23.53 536/23.6 536/23.7 536/24.1 800/278 800/295 800/298. C12N016/00 A01H003/00 A01H005/00.
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- ☒ 14. 6355240. 25 Jul 96; 12 Mar 02. Enhanced insecticidal insect virus through the expression of heterologous proteins with early promoters. Dierks; Peter M.. 424/93.2; 435/235.1 435/320.1 435/69.1. A01N063/00 C12N007/01 C12P021/00.
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- ☐ 15. 6342595. 14 Dec 99; 29 Jan 02. Oncoprotein protein kinase. Karin; Michael, et al. 536/23.5; 435/252.3 435/320.1 435/69.1. C12N015/63.
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- ☐ 16. 6303341. 14 May 99; 16 Oct 01. Method for producing immunoglobulins containing protection proteins in plants and their use. Hiatt; Andrew C., et al. 435/70.1; 435/320.1 435/419 435/468 435/69.1 536/23.5 536/23.53 536/24.1 536/24.3. C12M015/00 C12M015/29 C12M015/80 A01H004/00.
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- ☐ 17. 6258595. 23 Sep 99; 10 Jul 01. Compositions and methods for helper-free production of recombinant adeno-associated viruses. Gao; Guang-Ping, et al. 435/320.1; 435/239 435/325 435/455 435/466 514/44 536/23.1. C12N015/86 C12N015/00 C12N005/00 C07H021/04 A61K048/00.
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- ☐ 18. 6225112. 23 Jun 98; 01 May 01. Human p27Kip1 gene promoter. Sakai; Toshiyuki, et al. 435/320.1; 435/325 536/23.1 536/24.1. C12N015/63 C12N005/00 C07H021/04.
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- ☐ 19. 6222096. 01 Mar 99; 24 Apr 01. Promoter and construct for plant transformation. Held; Bruce Marvin, et al. 800/278; 435/320.1 435/418 435/419 435/468 435/69.1 536/24.1 800/279 800/286 800/290 800/298 800/300 800/301 800/302. C12N005/04 C12N015/09 C12N015/29 C12N015/82 C12N015/90.
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- ☐ 20. 6207817. 07 Oct 99; 27 Mar 01. Fish insulin-like growth factor II promoter. Wu; Jen-Leih, et al. 536/24.1; 435/320.1 435/455 800/25 800/3. C07H021/04 C12N015/00 C12N015/63 C12Q001/68.
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- ☐ 21. 6200760. 13 Aug 99; 13 Mar 01. Method of screening agents as candidates for drugs or sources of drugs. Dannenberg; Andrew J., et al. 435/6; 435/4 435/471 435/476 435/8 435/91.1. C12Q001/68 C12Q001/66 C12P019/34.
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- ☐ 22. 6193965. 30 Nov 99; 27 Feb 01. Oncoprotein kinase. Karin; Michael, et al. 424/131.1; 424/139.1 514/12. A61K038/16 A61K039/395.
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- ☒ 23. 6118049. 18 Sep 98; 12 Sep 00. Synthetic hybrid tomato E4/E8 plant promoter. Bestwick; Richard K., et al. 800/283; 435/320.1 435/419 536/24.1. C07H021/04 C12N005/14 C12N015/82.
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- ☐ 24. 6110744. 12 Nov 97; 29 Aug 00. Diminishing viral gene expression by promoter replacement. Fang; Bingliang, et al. 435/456; 435/320.1 536/23.1 536/23.5 536/23.7 536/23.72 536/24.1. C12N015/64 C12N015/34 C12N015/12.
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- ☐ 25. 6107037. 19 Jun 98; 22 Aug 00. Methods for using mutant RNA polymerases with reduced discrimination between non-canonical and canonical nucleoside triphosphates. Sousa; Rui, et al. 435/6; 435/5 435/91.2 536/22.1 536/24.3 536/24.32. C12Q001/68 C12P019/34 C07H021/04.
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- ☒ 26. 6072050. 24 Feb 98; 06 Jun 00. Synthetic promoters. Bowen; Benjamin A., et al. 536/24.1; 435/320.1 435/419 435/468 536/23.6 536/24.2 800/278 800/295 800/298. A01H005/00 A01H004/00

C12N015/00 C12N015/29 C12N015/82.

☐ 27. 6046037. 04 May 95; 04 Apr 00. Method for producing immunoglobulins containing protection proteins in plants and their use. Hiatt; Andrew C., et al. 435/70.1; 435/320.1 435/419 435/468 435/69.1 435/69.7 536/23.5 536/23.53 536/24.1 536/24.3 800/278. C12N015/00 C12N015/29 C12N015/82 A01H004/00.

☐ 28. 6018040. 20 Jul 98; 25 Jan 00. Fish insulin-like growth factor 11 promoter. Wu; Jen-Lieh, et al. 536/24.1; 435/320.1 536/23.1 800/25. C07H021/02 C07H021/04 C12N015/00.

☐ 29. 6001584. 08 Sep 98; 14 Dec 99. Oncoprotein protein kinase. Karin; Michael, et al. 435/15; 435/975. C12Q001/48.

☐ 30. 5994513. 08 Sep 98; 30 Nov 99. Oncoprotein protein kinase. Karin; Michael, et al. 530/387.9; C07K016/40.

☐ 31. 5959177. 03 May 96; 28 Sep 99. Transgenic plants expressing assembled secretory antibodies. Hein; Mich B., et al. 800/288; 435/320.1 435/419 435/69.1 536/23.5 536/23.53 536/24.1 800/295. C12N015/00 C12N015/29 C12N015/82 A01H005/00.

☒ 32. 5945304. 18 Jul 97; 31 Aug 99. Expression plasmids regulated by an osmB promoter. Fischer; Meir. 435/69.1; 435/252.33 435/320.1 536/24.1. C12P021/02 C07H021/04 C12N001/21 C12N015/70.

☐ 33. 5891631. 14 Jun 96; 06 Apr 99. Methods relating tosterol regulatory element binding proteins. Goldstein; Joseph L., et al. 435/6; 435/367 435/369 435/7.1. C12Q001/68 G01N033/53.

☐ 34. 5886019. 07 Jun 95; 23 Mar 99. Protein kinase C modulators. F.. Driedger; Paul E., et al. 514/410; 514/183 514/63 514/81 540/451 540/460 540/461. A61K031/395 A61K031/40 C07D487/00 C07D487/06.

☐ 35. 5871742. 25 Sep 95; 16 Feb 99. Recombinant Avipox virus encoding polypeptide of mycoplasma gallisepticum, and utilized a live vaccine. Saitoh; Shuji, et al. 424/199.1; 424/190.1 424/192.1 424/214.1 424/215.1 424/232.1 424/264.1 435/320.1 435/69.1 435/69.3 435/70.1 530/350 530/820 530/825 536/23.4 536/23.7 930/200. A61K039/295 A61K039/12 C12N015/31 C07K007/01.

☐ 36. 5869298. 03 Apr 95; 09 Feb 99. Stable mutants of D-N-.alpha.-carbamoylase and process for preparing D-.alpha.-amino acids. Galli; Giuliano, et al. 435/106; 435/195 435/78. C12P013/04 C12P019/56 C12N009/14.

☐ 37. 5849546. 13 Sep 96; 15 Dec 98. Methods for using mutant RNA polymerases with reduced discrimination between non-canonical and canonical nucleoside triphosphates. Sousa; Rui, et al. 435/91.5; 435/91.2 536/24.3 536/24.5. C07H021/04 C12P019/34.

☐ 38. 5837244. 12 Sep 96; 17 Nov 98. Oncoprotein protein kinase. Karin; Michael, et al. 424/139.1; 435/194 514/44. A61K031/90 A61K039/395.

☒ 39. 5824469. 30 Sep 94; 20 Oct 98. Method for producing novel DNA sequences with biological activity. Horwitz; Marshall S., et al. 435/6; 435/488 435/91.1 536/23.1 536/24.1. C12Q001/68 C12N015/11 C12N015/10 C12P019/34.

- ☐ 40. 5807710. 25 Jul 97; 15 Sep 98. Nucleic acids encoding stable mutants of D-N-.alpha.-carbamoylase. Galli; Giuliano, et al. 435/69.1; 435/183 435/320.1 435/325 536/23.2. C12P021/06 C12N009/00 C07H017/00.
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- ☐ 41. 5804399. 13 Feb 97; 08 Sep 98. Oncoprotein protein kinase. Karin; Michael, et al. 435/15; 435/194. C12Q001/48.
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- ☐ 42. 5795776. 22 Mar 94; 18 Aug 98. Expression plasmids regulated by an OSMB promoter. Fischer; Meir. 435/320.1; 435/192 435/252.33 435/69.1 435/69.3 435/69.4 536/24.1. C12N015/70 C12N015/63 C12P021/02 C07H021/04.
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- ☐ 43. 5721137. 08 Mar 95; 24 Feb 98. Plasmid vector and its use for the production of heterologous proteins. Frascotti; Gianni, et al. 435/320.1; 435/252.31 435/252.33 435/69.1. C12N015/70 C12N015/75 C12N015/11 C12P021/02.
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- ☐ 44. 5639947. 05 Nov 92; 17 Jun 97. Compositions containing glycopolypeptide multimers and methods of making same in plants. Hiatt; Andrew C., et al. 800/267; 435/69.6 530/387.1 530/387.3 536/23.53 800/288 800/298. A01H005/00 C12N015/13 C12N015/82.
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- ☐ 45. 5633441. 22 Dec 94; 27 May 97. Plants with genetic female sterility. De Greef; Willy, et al. 800/271; 435/320.1 435/418 435/419 536/24.1 800/287 800/317.3. A01H005/00 C12N015/11 C12N015/82.
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- ☐ 46. 5605808. 19 May 95; 25 Feb 97. Oncoprotein protein kinase. Karin; Michael, et al. 435/15; 435/194. C12Q001/48.
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- ☐ 47. 5599904. 03 Mar 92; 04 Feb 97. Chimeric steroid hormone superfamily receptor proteins. Evans; Ronald M., et al. 530/350; 435/69.1 435/69.7. C07K019/00 C07K014/705.
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- ☐ 48. 5593884. 18 Jul 94; 14 Jan 97. Oncoprotein protein kinase. Karin; Michael, et al. 435/252.3; 435/194 435/320.1 536/23.2. C12N015/54 C12N015/74.
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- ☐ 49. 5571692. 16 Dec 93; 05 Nov 96. Retinoic acid receptor .alpha., vectors and cells comprising the same DNA encoding. Evans; Ronald M., et al. 435/69.1; 435/252.3 435/254.11 435/320.1 435/364 536/23.5. C12N015/12 C12N015/63 C12N005/10 C12N001/21.
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- ☐ 50. 5548063. 11 Jan 94; 20 Aug 96. Retinoic acid receptor alpha proteins. Evans; Ronald M., et al. 530/350; 435/69.1 530/324. C07K014/705.
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[Generate Collection](#)[Print](#)

Terms	Documents
19 with L12	77

[Previous Page](#)[Next Page](#)

09/782200
Att#6

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=> s promoter?
L1 409592 PROMOTER?

=> s (activity or activities)(5n)(increas?)
4 FILES SEARCHED...
L2 562747 (ACTIVITY OR ACTIVITIES)(5N)(INCREAS?)

=> s (activity or activities)(5n)(compar?)
L3 172214 (ACTIVITY OR ACTIVITIES)(5N)(COMPAR?)

=> s (activity or activities)(5n)(improv?)
L4 34492 (ACTIVITY OR ACTIVITIES)(5N)(IMPROV?)

=> s l2 or l3 or l4
L5 735078 L2 OR L3 OR L4

=> s l1 and l5
L6 20952 L1 AND L5

=> s l1(l)l5
L7 19134 L1(L) L5

=> s hybrid or hybrids or mutat?
L8 1140022 HYBRID OR HYBRIDS OR MUTAT?

=> s l1 and l5 and l8
L9 4554 L1 AND L5 AND L8

=> s l7 and l8
L10 4366 L7 AND L8

=> s fusion? or join? or link?
L11 2820781 FUSION? OR JOIN? OR LINK?

=> s l7 and l8 and l11
L12 1044 L7 AND L8 AND L11

=> s l1(l)l8
L13 62348 L1(L) L8

=> s l7 and l13 and l11
L14 940 L7 AND L13 AND L11

=> s l1(5n)l5
L15 6202 L1(5N) L5

=> s l1(5n)l8
L16 14360 L1(5N) L8

=> s l15 and l16 and l11
L17 101 L15 AND L16 AND L11

=> rd
RD IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> rd l17
RD IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> l7
l7 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> l17
L17 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> help commands
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Enter one of these commands at the arrow prompt (=>).

ACTIVATE ----- Assign L#s to saved query or answer set.
 ANALYZE ----- Build expand terms from answer fields.
 ARCHIVE ----- Purchase rights for archiving.
 DELETE ----- Delete saved or current session items.
 DISPLAY ----- Display saved or current session items.
 DUPLICATE ----- Determine duplicate answers
 EDIT ----- Modify the text of an E-number entry.
 EXPAND ----- Look at the index around a term.
 FILE ----- Specify the search and display file.
 FOCUS ----- Rank answers in order of relevancy.
 FSEARCH ----- Find records from given patent family(s)
 FSORT ----- Sort patent records by patent family
 HELP ----- For help on how to use the system.
 INDEX ----- Specify the Index environment.
 LOGOFF ----- End the online session.
 NEWS ----- Display current news about the system.
 PRINT ----- Print answers offline.
 REDISTRIBUTE - Purchase rights for redistribution.
 SAVE ----- Save an L-numbered query or answer set.
 SDI ----- Request searches be run on file updates.
 SEARCH ----- Perform a search.
 SELECT ----- Build expand terms from answer fields.
 SET ----- Set terminal and interaction options.
 SORT ----- Sort Answers of an L-number list
 TABULATE ----- Analyze SmartSELECT L-number data.
 TRANSFER ----- Extract terms from answers and search.
 ? ----- The same as HELP.

Enter one of these commands at the arrow prompt (=>).

For more detailed information about a command, enter HELP followed by the command name at an arrow prompt.

Example: => HELP SEARCH

For information on entering multiple commands at a single arrow prompt, enter HELP COMMAND STACKING.

```
=> display
ENTER (L17), L# OR ? : l1
ENTER ANSWER NUMBER OR RANGE (1): l1-l2
ANSWER NUMBERS NOT CORRECTLY SPECIFIED
Enter an answer number, Example: 10
several answer numbers, Example: 3,7,10
a range of answer numbers, Example: 5-10
or a combination of these. Example: 3,7,9-10,15
ENTER ANSWER NUMBER OR RANGE (1): 1-2
ENTER DISPLAY FORMAT (FILEDEFAULT): ?
Take the default to display records in the default format for each
file, or choose another display field or format. The field or format
you choose must be valid for all files in the multifile session.
Predefined display formats such as BIB, ABS, IND, and ALL exist in
almost all files.
```

For information on display fields or formats for a specific file of the multifile session, enter END to exit the DISPLAY command. Then at the arrow prompt (=>), enter HELP DFIELD FILE= followed by the file name, e.g., HELP DFIELD FILE=CAPLUS, or HELP FORMATS FILE= followed by the name, e.g., HELP FORMAT FILE=COMPENDEX. ENTER DISPLAY FORMAT (FILEDEFAULT): bib

```
L1 ANSWER 1 OF 409592 BIOSIS COPYRIGHT 2002 BIOSIS
AN 2002:52536 BIOSIS
DN PREV200200052536
TI Benzopyran derivatives and their use.
AU Sohda, T.; Taketomi, S.; Oda, T.
CS Takatsuki Japan
ASSIGNEE: TAKEDA CHEMICAL INDUSTRIES, LTD.
PI US 5580863 Dec. 3, 1996
SO Official Gazette of the United States Patent and Trademark Office
Patents,
(Dec. 3, 1996) Vol. 1193, No. 1, pp. 448-449.
ISSN: 0098-1133.
DT Patent
LA English
```

L1 ANSWER 2 OF 409592 BIOSIS COPYRIGHT 2002 BIOSIS
AN 2002:51845 BIOSIS
DN PREV200200051845
T1 Vectors with pectate lyase signal sequence.
AU Robinson, R. R.; Better, M.; Lei, S-P.; Wilcox, G. L.
CS Los Angeles, Calif. USA
ASSIGNEE: KOMA CORPORATION
PI US 5576195 Nov. 19, 1996
SO Official Gazette of the United States Patent and Trademark Office
Patents,
(Nov. 19, 1996) Vol. 1192, No. 3, pp. 2028.
ISSN: 0098-1133.
DT Patent
LA English

=> d his

(FILE 'HOME' ENTERED AT 10:01:59 ON 05 JAN 2002)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED
AT 10:02:11 ON 05
JAN 2002

L1 409592 S PROMOTER?
L2 562747 S (ACTIVITY OR ACTIVITIES)(5N)(INCREAS?)
L3 172214 S (ACTIVITY OR ACTIVITIES)(5N)(COMPAR?)
L4 34492 S (ACTIVITY OR ACTIVITIES)(5N)(IMPROV?)
L5 735078 S L2 OR L3 OR L4
L6 20952 S L1 AND L5
L7 19134 S L1(L)L5
L8 1140022 S HYBRID OR HYBRIDS OR MUTAT?
L9 4554 S L1 AND L5 AND L8
L10 4366 S L7 AND L8
L11 2820781 S FUSION? OR JOIN? OR LINK?
L12 1044 S L7 AND L8 AND L11
L13 62348 S L1(L)L8
L14 940 S L7 AND L13 AND L11
L15 6202 S L1(5N)L5
L16 14360 S L1(5N)L8
L17 101 S L15 AND L16 AND L11

=> rd l17

RD IS NOT A RECOGNIZED COMMAND

The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> dup rem l17

PROCESSING COMPLETED FOR L17

L18 43 DUP REM L17 (58 DUPLICATES REMOVED)

=> d l18 ibib abs 1-43

L18 ANSWER 1 OF 43 WPIDS COPYRIGHT 2002 DERWENT
INFORMATION LTD

ACCESSION NUMBER: 2001-123321 [13] WPIDS

DOC. NO. CPI: C2001-035890

TITLE: New isolated mutated human p53 polypeptides for inducing
toxicity in a cell, treating cancer and identifying
compounds that mimic toxic or supertransactivating
mutations.

DERWENT CLASS: B04 D16

INVENTOR(S): INGA, A; RESNICK, M A

PATENT ASSIGNEE(S): (USSH) US DEPT HEALTH & HUMAN
SERVICES

COUNTRY COUNT: 94

PATENT INFORMATION:

PATENT NO KIND DATE WEEK LA PG

WO 2001009325 A2 20010208 (200113)* EN 144

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE
LS LU MC MW MZ

NL OA PT SD SE SL SZ TZ UG ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN
CR CU CZ DE DK DM

DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KP KR KZ LC

LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ
PL PT RO RU SD SE
SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000062395 A 20010219 (200129)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001009325 A2		WO 2000-US20538	20000728
AU 2000062395 A		AU 2000-62395	20000728

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000062395 A	Based on	WO 200109325

PRIORITY APPLN. INFO: US 1999-146634P 19990730

AN 2001-123321 [13] WPIDS

AB WO 200109325 A UPAB: 20010307

NOVELTY - Isolated polypeptides (I) of human p53 containing specific
mutations, are new.

DETAILED DESCRIPTION - New isolated polypeptides (I) of p53
having

the following residues containing the mutations (in brackets):

(a) 117 to 172 (V122A);
(b) 272 to 282 (C277W);
(c) 272 to 282 (C277R);
(d) 333 to 343 (F338L);
(e) 153 to 163 (V157I);
(f) 70 to 80 (A76T);
(g) 145 to 155 (T150A);
(h) 115 to 125 (S121C);
(i) 90 to 100 (S96P);
(j) 110 to 120 (H115R);
(k) 120 to 130 (C124Y);
(l) 115 to 125 (S121F);
(m) 118 to 128 (T123A);
(n) 120 to 130 (C124F);
(o) 235 to 245 (S240N);
(p) 110 to 120 (S116T);
(q) 340 to 350 (N345S);
(r) 118 to 128 (T123S);
(s) 180 to 190 (D184G);
(t) 283 to 293 (N288K);
(u) 193 to 203 (E198V);
(v) 110 to 120 (H115R);
(w) 85 to 95 (W92R);
(x) 90 to 100 (S96P);
(y) 110 to 120 (S116T);
(z) 225 to 235 (N228K);
(a') 113 to 123 (T118A);
(b') 118 to 128 (T123P);
(c') 132 to 142 (L137R);
(d') 155 to 165 (M160T);
(e') 235 to 245 (N239Y);
(f') 280 to 290 (E285A);
(g') 50 to 150 (A76T) and (V22A);
(h') 50 to 200 (W91C), (C124R), (Q136K), and (T150A); or
(i') 100 to 200 (C124R), (Q136K) and (T150A).

INDEPENDENT CLAIMS are also included for the following:

(1) an isolated nucleic acid (II) encoding (I);
(2) detecting (M1) a supertransactivating mutation in (II)

comprising:

(a) introducing (II) comprising an inducible promoter containing GAL
1 linked to a human p53 coding sequence, into a yeast cell that has a
reporter gene linked to a DNA sequence that p53 binds;
(b) plating the cell on raffinose as a carbon source; and
(c) identifying colonies on plates where wild type and mutant
colonies yield different colored colonies;
(3) detecting (M2) a toxic mutation in the human p53 gene comprises
(M1), where the cell can be plated on glucose, raffinose or galactose;
(4) detecting (M3) a toxic mutation in the human p53 gene comprising:
(a) introducing a nucleic acid encoding an unidentified human p53 and
containing an on-off promoter linked to the coding sequence, into a yeast
cell;
(b) incubating the cell in synthetic yeast medium in the presence and

absence of an inducer for the promoter; and

(c) yeast expressing wildtype p53 yield grow in the presence or absence of the inducer and yeast expressing a mutation yield grow in the presence of the inducer;

(5) inducing (M4) toxicity in a cell by administering (I);

(6) screening (M5) for compounds that can mimic a toxic p53 mutation comprising:

(a) introducing into a yeast cell of (M1(a)), a nucleic acid that encodes a non-toxic or wildtype p53 and that contains an inducible promoter linked to the coding sequence;

(b) introducing the compound to the cell;

(c) plating the cell on glucose, raffinose or galactose;

(d) identifying a compound that mimics a toxic mutation preventing growth of colonies expressing wildtype or non-toxic mutant p53;

(7) screening (M6) for compounds that can mimic a toxic p53 mutation comprising:

(a) introducing into a yeast cell a nucleic acid which encodes a non-toxic mutant or wildtype p53 and comprising an on-off promoter linked to the coding sequence;

(b) introducing the compound to the cell;

(c) (M3(b)); and

(d) identifying a compound that mimics a toxic mutation, preventing growth of yeast in the presence of the inducer;

(8) screening (M7) for a compound that can mimic a supertransactivating mutation in the p53 gene comprising:

(a) introducing a nucleic acid into the yeast cell of (M1(a)), that encodes a wildtype or a non-supertransactivating mutant p53 and comprising an inducible promoter linked to the coding sequence;

(b) plating the yeast cell and compound on raffinose medium;

(c) identifying a compound that mimics a supertransactivating mutation in p53;

(9) determining (M8) transactivation by supertransactivating p53 mutants at different expression levels and with different p53 responsive elements comprising:

(a) plating two yeast cells with two different DNA sequences that bind p53, that have been through steps (a) and (b) of (M1) on glucose, raffinose, raffinose and galactose, and raffinose and more galactose;

(b) identifying colonies on plates, where white or pink colonies indicate transactivation has occurred; and

(c) determining the level of supertransactivation of the two DNA sequences under different levels of expression by p53;

(10) detecting (M9) a supertransactivating mutation in (II) comprising:

(a) obtaining a reg1-501 mutant yeast cell of (M1(a));

(b) plating the cell on glucose and glucose with increasing concentrations of galactose; and

(c) identifying colonies on plates due to color changes;

(11) detecting (M10) a toxic mutation in (II) comprising (M9); and

(12) identifying a p53 mutant with weak transactivating activity by introducing a nucleic acid encoding p53 and comprising a promoter such as antidiuretic hormone 1 (ADH1) into a yeast cell that contains a reporter gene, plating the cell on glucose and identifying colonies due to color changes.

ACTIVITY - Cytostatic. No biological data is given.

MECHANISM OF ACTION - Gene therapy.

USE - (I) are used to induce toxicity in a cell (claimed). Mutants of p53 that are toxic, supertransactivating, or tox-suppressing are identified. (I) are used to identify compounds, agents or interactive factors, such as peptides, that mimic the toxic or supertransactivating mutations (claimed). (I) can be used to inhibit growth and treat cancer.

Dwg.0/18

L18 ANSWER 2 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 1
 ACCESSION NUMBER: 2001:127592 BIOSIS
 DOCUMENT NUMBER: PREV200100127592
 TITLE: The rainbow trout metallothionein-B gene promoter: Contributions of distal promoter elements to metal and oxidant regulation.
 AUTHOR(S): Samson, Susan L.-A.; Paramchuk, Wendy J.; Gedamu, Lashitew
 (1)
 CORPORATE SOURCE: (1) Department of Biological Sciences, University of Calgary, 2500 University Drive N.W., Calgary, Alberta, T2N

IN4: lgedamu@ucalgary.ca Canada
 SOURCE: Biochimica et Biophysica Acta, (26 January, 2001) Vol. 1517, No. 2, pp. 202-211. print.
 ISSN: 0006-3002.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 SUMMARY LANGUAGE: English
 AB In this report, the contributions of the distal 5'-regulatory sequences of the rainbow trout (*Oncorhynchus mykiss*) metallothionein (tMT)-B gene promoter (-738 to +5) were studied. Transfection of the -738 promoter fragment in a rainbow trout hepatoma cell line (RTH-149) resulted in 4- to 5-fold greater ***activity*** ***compared*** to the proximal -137 ***promoter*** region. ***Mutation*** of the proximal MREa abolishes the basal activity of the -738 fragment indicating that the distal regulatory elements require a cooperative interaction with MREa. However, the fragments containing both distal MREs, c and d (positioning -570 and -680, respectively), or MREc alone could confer basal and metal-induced activity when fused to the TATA box. This suggests that these distal elements are functional and therefore may play a role as basal elements in their natural state. The trout MT genes are also induced by oxidants including H2O2, tBHP and tBHQ. The larger promoter fragment -738 responds to H2O2, while the -137 fragment does not. However, ***fusion*** of the isolated MREc fragment (-648 to -533) in its native orientation, upstream of the -137 promoter elicits a response to H2O2, although no response is seen with MREc in reverse. These data suggest that this distal fragment contains functional oxidant responsive elements which have resemblance to the mammalian antioxidant responsive element (AREs).

L18 ANSWER 3 OF 43 MEDLINE
 ACCESSION NUMBER: 2000266266 MEDLINE
 DOCUMENT NUMBER: 20266266 PubMed ID: 10805720
 TITLE: Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells.
 AUTHOR: Misiti S; Nanni S; Fontemaggi G; Cong Y S; Wen J; Hirte H
 W; Piaggio G; Sacchi A; Pontecorvi A; Bacchetti S; Farsetti A
 CORPORATE SOURCE: Molecular Oncogenesis Laboratory, Regina Elena Cancer Institute, Rome, Italy.
 SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (2000 Jun) 20 (11) 3764-71.
 Journal code: NGY; 8109087. ISSN: 0270-7306.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200007
 ENTRY DATE: Entered STN: 20000714
 Last Updated on STN: 20000714
 Entered Medline: 20000706
 AB In mammals, molecular mechanisms and factors involved in the tight regulation of telomerase expression and activity are still largely undefined. In this study, we provide evidence for a role of estrogens and their receptors in the transcriptional regulation of hTERT, the catalytic subunit of human telomerase and, consequently, in the activation of the enzyme. Through a computer analysis of the hTERT 5'-flanking sequences, we identified a putative estrogen response element (ERE) which was capable of binding in vitro human estrogen receptor alpha (ERalpha). In vivo DNA footprinting revealed specific modifications of the ERE region in ERalpha-positive but not ERalpha-negative cells upon treatment with 17beta-estradiol (E2), indicative of estrogen-dependent chromatin remodelling. In the presence of E2, transient expression of ERalpha but not ERbeta remarkably ***increased*** hTERT ***promoter*** ***activity***, and ***mutation*** of the ERE significantly reduced this effect. No telomerase activity was detected in human ovary epithelial cells grown in the absence of E2, but the addition of the hormone induced the enzyme within 3 h of treatment. The expression of hTERT mRNA and protein was induced in parallel with enzymatic activity. This prompt estrogen modulation of telomerase activity substantiates estrogen-dependent transcriptional regulation of the hTERT gene. The identification of hTERT as a target of estrogens represents a novel finding which advances the understanding of telomerase regulation in

hormone-dependent cells and has implications for a potential role of hormones in their senescence and malignant conversion.

L18 ANSWER 4 OF 43 MEDLINE

ACCESSION NUMBER: 200011280 MEDLINE

DOCUMENT NUMBER: 20111280 PubMed ID: 10644332

TITLE: The human immunodeficiency virus type 1 Tat protein up-regulates the promoter activity of the beta-chemokine monocyte chemoattractant protein 1 in the human astrocytoma cell line U-87 MG: role of SP-1, AP-1, and NF-kappaB consensus sites.

AUTHOR: Lim S P; Garzino-Demo A

CORPORATE SOURCE: Institute of Molecular and Cell Biology, Singapore 117609, Singapore.

SOURCE: JOURNAL OF VIROLOGY, (2000 Feb) 74 (4) 1632-40.

Journal code: KCV; 0113724. ISSN: 0022-538X.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200003

ENTRY DATE: Entered STN: 20000314

Last Updated on STN: 20000314

Entered Medline: 20000302

AB It has been shown that the human immunodeficiency virus type 1 (HIV-1) Tat

protein can specifically enhance expression and release of monocyte chemoattractant protein 1 (MCP-1) from human astrocytes. In this study, we

show evidence that Tat-induced MCP-1 expression is mediated at the transcriptional level. Transient transfection of an expression construct encoding the full-length Tat into the human glioblastoma-astrocytoma cell line U-87 MG enhances reporter gene activity from cotransfected deletion constructs of the MCP-1 promoter. HIV-1 Tat exerts its effect through a minimal construct containing 213 nucleotides upstream of the translational start site. Site-directed mutagenesis studies indicate that an SP1 site (located between nucleotides -123 and -115) is critical for both constitutive and Tat-enhanced expression of the human MCP-1 ***promoter***, as ***mutation*** of this SP1 site significantly diminished reporter gene expression in both instances. Gel retardation experiments further demonstrate that Tat strongly enhances the binding of SP1 protein to its DNA element on the MCP-1 ***promoter***.

Moreover,

we also observe an ***increase*** in the binding ***activities*** of transcriptional factors AP1 and NF-kappaB to the MCP-1 promoter following Tat treatment. Mutagenesis studies show that an upstream AP1 site and an adjacent NF-kappaB site (located at -128 to -122 and -150 to -137, respectively) play a role in Tat-mediated transactivation. In contrast, a further upstream AP1 site (-156 to -150) does not appear to be crucial for promoter activity. We postulate that a Tat-mediated increase in SP1 binding activities augments the binding of AP1 and NF-kappaB, leading to synergistic activation of the MCP-1 promoter.

L18 ANSWER 5 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
DUPLICATE 2

ACCESSION NUMBER: 2001:35862 BIOSIS

DOCUMENT NUMBER: PREV200100035862

TITLE: Molecular regulation of constitutive expression of interleukin-8 in human pancreatic adenocarcinoma.

AUTHOR(S): Le, Xiangdong; Shi, Qian; Wang, Bailiang; Xiong, Qinghua;

Qian, Chaonan; Peng, Zhihai; Li, Xiang-Cheng; Tang, Huamei; Abbruzzese, James L.; Xie, Keping (1)

CORPORATE SOURCE: (1) Department of Gastrointestinal Oncology and Digestive

Diseases, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX, 77030; kepxie@notes.mdacc.tmc.edu USA

SOURCE: Journal of Interferon and Cytokine Research, (November, 2000) Vol. 20, No. 11, pp. 935-946. print. ISSN: 1079-9907.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Recent studies have shown that interleukin-8 (IL-8) plays an important role in the growth and metastasis of human pancreatic cancer. In the present study, we determined the molecular regulation of constitutive IL-8

expression in human pancreatic cancer cells. Various human pancreatic cancer cell lines were incubated in vitro. Sixty-seven percent of the cell lines constitutively secreted high levels of IL-8, as determined using enzyme- ***linked*** immunosorbent assay. Consistently, these cells constitutively expressed high levels of IL-8 mRNA, as determined using Northern blot analysis. To determine the mechanisms of the high steady-state levels of IL-8 mRNA, the IL-8 half-life and transcription rate were measured. There was no significant difference in IL-8 half-life between cells expressing high and low levels of IL-8. However, higher transcription rates and ***increased*** IL-8 ***promoter*** ***activity*** were observed in the cells constitutively expressing high levels of IL-8. Detailed IL-8 ***promoter*** analysis using deletion ***mutation*** revealed that the region from -85 to -133 bp was essential for the constitutive IL-8 ***promoter*** activity. Also, point- ***mutation*** analysis indicated that mutation of NF-kappaB, AP-1, or NF-IL-6 binding sites significantly reduced or eliminated the constitutive IL-8 promoter activity. Consistent with the constitutive IL-8 transcription activity, high levels of constitutive NF-kappaB and AP-1 activity were detected in the cells overexpressing IL-8, as determined using electrophoretic mobility shift assay. In addition, transfection of a dominant-negative I-kappaBalpha expression vector (I-kappaBalphaM) inhibited constitutive NF-kappaB activity and IL-8 expression in pancreatic cancer cells. Collectively, our data demonstrated that constitutive NF-kappaB and AP-1 activation contributes to the overexpression of IL-8, which in turn plays an important role in tumor angiogenesis and contributes to the aggressive biology of human pancreatic cancer.

L18 ANSWER 6 OF 43 MEDLINE

ACCESSION NUMBER: 2001064151 MEDLINE

DOCUMENT NUMBER: 20431046 PubMed ID: 10976534

TITLE: Level of interleukin-8 expression by metastatic human melanoma cells directly correlates with constitutive NF-kappaB activity.

AUTHOR: Huang S; DeGuzman A; Bucana C D; Fidler I J

CORPORATE SOURCE: Department of Cancer Biology, The University of Texas MD

Anderson Cancer Center, Houston 77030, USA.

CONTRACT NUMBER: CA16672 (NCI)

R35-CA42107 (NCI)

SOURCE: CYTOKINES, CELLULAR AND MOLECULAR THERAPY, (2000 Mar) 6 (1) 9-17.

Journal code: CUS. ISSN: 1368-4736.

PUB. COUNTRY: ENGLAND: United Kingdom

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200012

ENTRY DATE: Entered STN: 20010322

Last Updated on STN: 20010322

Entered Medline: 20001222

AB The purpose of this study was to determine whether constitutive NF-kappaB

activity regulates the expression level of interleukin-8 (IL-8) in metastatic human melanoma cells. Cultures of metastatic human A375 melanoma cells expressed higher levels of IL-8 mRNA and protein than nonmetastatic A375 human melanoma cells. No discernible differences in IL-8 half-life were found between metastatic and nonmetastatic cells, but cells that overexpressed IL-8 had a higher transcription rate and ***increased*** IL-8 ***promoter*** ***activity***. Analysis of the IL-8 ***promoter*** using deletion mutants revealed that the region within -133 was essential for constitutive IL-8 ***promoter*** activity and that ***mutation*** of NF-kappaB binding sites eliminated the constitutive IL-8 promoter activity. The activation of constitutive IL-8 transcription directly correlated with the level of constitutive NF-kappaB activity. Transfection of melanoma cells with a dominant-negative mutant IkappaBalpha expression vector (pLXSN-IkappaBalphaM) significantly decreased the level of constitutive NF-kappaB activity and expression of IL-8, demonstrating that constitutive

NF-kappaB/relA activities contribute to overexpression of IL-8 in highly metastatic human melanoma cells.

L18 ANSWER 7 OF 43 WPIDS COPYRIGHT 2002 DERWENT
INFORMATION LTD

ACCESSION NUMBER: 1999-229526 [19] WPIDS

DOC. NO. CPI: C1999-067559
 TITLE: ***Hybrid*** ***promoter*** containing parts of
 the tomato E4 and E8 promoters.
 DERWENT CLASS: C06 D16
 INVENTOR(S): BESTWICK, R K; KELLOGG, J A
 PATENT ASSIGNEE(S): (EXEL-N) EXELIXIS PLANT SCI INC;
 (AGRI-N) AGRITOPIC INC
 COUNTRY COUNT: 83
 PATENT INFORMATION:

PATENT NO KIND DATE WEEK LA PG

 WO 9914316 A2 19990325 (199919)* EN 52
 RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE
 LS LU MC MW NL
 OA PT SD SE SZ UG ZW
 W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE
 DK EE ES FI GB GE
 GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT
 LU LV MD MG
 MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ
 TM TR TT UA UG
 US UZ VN YU ZW
 AU 9910611 A 19990405 (199933)
 EP 1012318 A2 20000628 (200035) EN
 R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV
 MC MK NL PT
 RO SE SI
 US 6118049 A 20000912 (200046)
 AU 737124 B 20010809 (200152)
 NZ 503791 A 20010831 (200157)
 JP 2001516576 W 20011002 (200172) 66

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9914316	A2	WO 1998-US19571	19980918
AU 9910611	A	AU 1999-10611	19980918
EP 1012318	A2	EP 1998-953173	19980918
		WO 1998-US19571	19980918
US 6118049	A	Provisional US 1997-59234P	19970918
		US 1998-157077	19980918
AU 737124	B	AU 1999-10611	19980918
NZ 503791	A	NZ 1998-503791	19980918
		WO 1998-US19571	19980918
JP 2001516576 W		WO 1998-US19571	19980918
		JP 2000-511856	19980918

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9910611	A Based on	WO 9914316
EP 1012318	A2 Based on	WO 9914316
AU 737124	B Previous Publ.	AU 9910611
	Based on	WO 9914316
NZ 503791	A Based on	WO 9914316
JP 2001516576 W	Based on	WO 9914316

PRIORITY APPLN. INFO: US 1997-59234P 19970918; US 1998-157077 19980918

AN 1999-229526 [19] WPIDS

AB WO 9914316 A UPAB: 19990518

NOVELTY - DNA construct (A) contains:

(1) a ***hybrid*** ***promoter*** containing parts of the tomato E4 and E8 promoters; and
 (2) heterologous DNA (I), and the ***hybrid*** ***promoter*** drives expression of a reporter gene with at least 75-300% of the expression level achieved with unmodified E4 or E8 promoters.

DETAILED DESCRIPTION - The ***hybrid*** ***promoter*** contains:

(1) at least 30 contiguous nucleotides (nt) from the 1-1395 nt region of a 2298 bp sequence (I) given in the specification, (encoding the E8 component), and
 (2) at least 200 contiguous nt from the 271-1437 nt region of a 2796 bp sequence (II) given in the specification, (encoding the E4 component).

INDEPENDENT CLAIMS are also included for the following:

(1) plant transformation vectors containing (A);
 (2) dicotyledonous cells transformed with (A); and
 (3) method for ***increasing*** ***activity*** of the tomato E4 ***promoter*** by fusing it to the specified E8 component.

ACTIVITY - None given.

MECHANISM OF ACTION - None given.

USE - (A) are used to generate transgenic plants, especially cucurbits, raspberries, strawberries and tomato, specifically melon, that express (I) that delays ripening of fruit.

Alternatively (I) is a pathogenesis-related gene; an antisense or co-suppression molecule or any other protein that modulates e.g. flowering, flavor, color, enzymes, plant hormones etc.

ADVANTAGE - The ***hybrid*** ***promoters*** provide higher

expression of heterologous proteins, in a fruit-specific manner, than either unmodified E4 or E8 promoters alone, and are functional in plants other than tomato. Vector pAG-7162, containing the

S-adenosylmethionine

hydrolase (SMase) gene under control of a E4/E8 ***hybrid***

promoter (nucleotides 1-1156 of E8 and 271-1437 of E4) was used to

transform melon (Cucumis melo through Agrobacterium, and transformed plants grown to develop fruit. Fruit pericarp was shown to contain significantly higher levels of SMase than the same tissue from fruits transformed with a vector containing either of the native promoters.

Dwg. 0/12

L18 ANSWER 8 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS

DUPLICATE 3

ACCESSION NUMBER: 2000:41893 BIOSIS

DOCUMENT NUMBER: PREV20000041893

TITLE: Basic fibroblast growth factor decreases elastin gene transcription through an AP1/cAMP-response element ***hybrid*** site in the distal ***promoter***

AUTHOR(S): Rich, Celeste B.; Fontanilla, Marta R.; Nugent, Matthew;

Foster, Judith Ann (1)

CORPORATE SOURCE: (1) Dept. of Biochemistry, Boston University School of

Medicine, 80 E. Concord St., Boston, MA USA

SOURCE: Journal of Biological Chemistry, (Nov. 19, 1999) Vol. 274,

No. 47, pp. 33433-33439.

ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Previous studies demonstrated that basic fibroblast growth factor (bFGF)

decreases elastin gene transcription in pulmonary fibroblasts. In this study we pursue the identification of the element and the trans-acting factors responsible. Gel shift analyses show that bFGF increases protein binding to a sequence located at -564 to -558 base pairs (bp), which possesses homology to both AP1 and cAMP-response consensus elements yet

displays a unique affinity for heterodimer binding. Site-directed mutation of the -564- to -558-bp sequence results in an ***increase*** in ***promoter*** ***activity*** and abrogates the effect of bFGF. Western blot analysis shows that bFGF induces a sustained increase in the steady-state levels of Fra 1, and co-transfection of a Fra 1 expression vector with an elastin promoter reporter construct results in an inhibition of elastin promoter activity. Overall the results suggest that bFGF represses elastin gene transcription by increasing the amount of the Fra 1 that subsequently binds to the -564- to -558-bp as a heterodimer with c-Jun to form an inhibitory complex. We propose that the identified bFGF response element can serve to down-regulate elastin transcription in elastogenic cells and, conversely, can serve to up-regulate elastogenesis in cells where endogenous bFGF signaling is attenuated or altered.

L18 ANSWER 9 OF 43 MEDLINE

ACCESSION NUMBER: 2000027378 MEDLINE

DOCUMENT NUMBER: 20027378 PubMed ID: 10557079

TITLE: The EGF/TGFalpha response element within the TGFalpha promoter consists of a multi-complex regulatory element.

AUTHOR: Awwad R; Humphrey L E; Periyasamy B; Scovell W Jr; Li W;

Coleman K; Lynch M; Carboni J; Brattain M G; Howell G M

CORPORATE SOURCE: Department of Biochemistry, Medical College of

Ohio, PO Box
10008, Toledo, Ohio, OH 43699-0008, USA.
CONTRACT NUMBER: CA34432 (NCI)
CA54807 (NCI)
SOURCE: ONCOGENE, (1999 Oct 21) 18 (43) 5923-35.
Journal code: ONC; 8711562. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991210

AB Autocrine TGFalpha is an important growth effector in the transformed phenotype. Growth stimulation of some colon cancer cells as well as other types of cancer cells is effected by activation of the epidermal growth factor receptor. Importantly, this receptor activation leads to further stimulation of TGFalpha transcription and increased peptide synthesis. However, the molecular mechanism by which TGFalpha transcription is activated is poorly understood. In this paper, we describe the localization of a cis-sequence within the TGFalpha promoter which mediates this stimulation. This region contains parallel cis-acting elements which interact to regulate both basal and EGF-induced TGFalpha expression. The well differentiated colon carcinoma cell line designated FET was employed in these studies. It produces autocrine TGFalpha but requires exogenous EGF in the medium for optimal growth. Addition of EGF to FET cells maintained in the absence of EGF resulted in a 2 - 3-fold ***increase*** of both TGF ***promoter*** ***activity*** and endogenous TGFalpha mRNA at 4 h. This addition of EGF also stimulated protein synthesis. The use of deletion constructs of the TGFalpha promoter in chimeras with chloramphenicol acetyl transferase localized EGF-responsiveness to between -247 and -201 within the TGFalpha promoter. A 25 bp sequence within this region conferred EGF-responsiveness to heterologous ***promoter*** constructs. Further use of deletion/ ***mutation*** chimeric constructs revealed the presence of at least two interacting cis-elements, one binding a repressor activity and the other, an activator. Gel shift studies indicate the presence of distinct complexes representing activator and repressor binding, which are positively modulated by EGF. The type and amount of complexes formed by these proteins interact to regulate both the basal activity and EGF-responsiveness of the TGFalpha promoter. The interaction of an activator protein with an EGF-responsive repressor may serve to regulate the level of this progression-associated, transforming protein within tight limits.

L18 ANSWER 10 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
DUPLICATE 4
ACCESSION NUMBER: 1999:247857 BIOSIS
DOCUMENT NUMBER: PREV199900247857
TITLE: Effects of mutant p53 expression on human
15-lipoxygenase-promoter activity and murine
12/15-lipoxygenase gene expression: Evidence that
15-lipoxygenase is a mutator gene.
AUTHOR(S): Kelavkar, Uddhav P.; Badr, Kamal F. (1)
CORPORATE SOURCE: (1) Center for Glomerulonephritis, 1840 Southern
Lane,
Decatur, GA, 30033 USA
SOURCE: Proceedings of the National Academy of Sciences of the
United States of America, (April 13, 1999) Vol. 96, No. 8,
pp. 4378-4383.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Human 15-lipoxygenase (h15-LO) is present on chromosome 17p13.3 in close proximity to the tumor-suppressor gene, p53. 15-LO is implicated in antiinflammation, membrane remodeling, and cancer development/metastasis.
The murine BALB/c embryo fibroblast cell line, (10)1val, expresses p53 in

mutant (mt) conformation when grown at 39degreeC and in wild-type conformation when grown at 32degreeC. Transfection of h15-LO promoter constructs (driving luciferase reporter) into (10)1val cells and into p53-deficient (10)1 cells resulted in a marked ***increase*** in h15-LO ***promoter*** ***activity*** in (10)1val cells at 39degreeC, but not at 32degreeC, or as compared with (10)1 cells. Transfection of h15-LO promoter deletion constructs, however, resulted in total loss of activity in both cell types at 32degreeC and 39degreeC. Cotransfection of (10)1 cells with h15-LO promoter (driving luciferase reporter) along with increasing levels of a mt p53 expression vector demonstrated dose-dependent capacity of mt p53 to induce 15-LO promoter activity. No effect was observed with wild-type p53. In contrast to h15-LO promoter activity, (10)1val cells had significantly lower levels of endogenous (murine) 12/15-LO (mouse analog of h15-LO) mRNA and protein when grown at 39degreeC compared with cells grown at 32degreeC. Our data support the hypothesis that loss of a tumor-suppressor gene (p53), or "gain-of-function activities" resulting from the expression of its mutant forms, regulates 15-LO promoter activity in man and in mouse, albeit in directionally opposite manners. The studies define a direct ***link*** between 15-LO activity and an established tumor-suppressor gene located in close chromosomal proximity.

L18 ANSWER 11 OF 43 MEDLINE
ACCESSION NUMBER: 2000047411 MEDLINE
DOCUMENT NUMBER: 20047411 PubMed ID: 10582586
TITLE: CTCF is essential for up-regulating expression from the amyloid precursor protein promoter during differentiation of primary hippocampal neurons.
AUTHOR: Yang Y; Quitschke W W; Vostrov A A; Brewer G J
CORPORATE SOURCE: Department of Medical Microbiology and Immunology, Southern Illinois University School of Medicine, Springfield 62794-9626, USA.
CONTRACT NUMBER: AG13435 (NIA)
NS30994 (NINDS)
SOURCE: JOURNAL OF NEUROCHEMISTRY, (1999 Dec) 73 (6) 2286-98.
Journal code: JAV; 2985190R. ISSN: 0022-3042.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991227

AB The transcriptional mechanism underlying amyloid precursor protein (APP) regulation in primary neurons during development was investigated. We observed an approximately threefold elevation of APP mRNA levels in differentiating rat hippocampal neurons between day 1 and day 7 in culture and in rat brain hippocampi between embryonic day 18 and postnatal day 3. When an APP promoter construct extending to position -2,832 upstream from the main transcriptional start site was transfected into primary rat hippocampal neurons, ***promoter*** ***activity*** ***increased*** from day 1 until reaching a maximum on day 7 in culture. This ***increase*** in APP ***promoter*** ***activity*** was correlated more closely with the time course of expression of the synaptic vesicle protein synaptophysin, an indicator of synaptogenesis, than with neurofilament accumulation, an indicator of neuritogenesis. Transfection of 5' APP ***promoter*** deletions and internal block ***mutations*** indicated that the CTCF binding domain designated APBBeta was the primary contributor to the ***increase*** in APP ***promoter*** ***activity***. Furthermore, the binding of transcription factor CTCF to the APBBeta element increased approximately fivefold between day 1 and day 7, whereas the binding of USF to the APBAlpha sequence increased only twofold. These results suggest that

CTCF

is pivotal for the up-regulation of APP expression during synaptogenesis in primary neurons.

L18 ANSWER 12 OF 43 MEDLINE DUPLICATE 5
 ACCESSION NUMBER: 2000062183 MEDLINE
 DOCUMENT NUMBER: 20062183 PubMed ID: 10597047
 TITLE: Functional characterization of TEL/AML1 ***fusion*** protein in the regulation of human CR1 gene promoter.
 AUTHOR: Song H; Kim J H; Rho J K; Park S Y; Kim C G; Choe S Y
 CORPORATE SOURCE: School of Life Sciences and Research Institute for Genetic Engineering, Chungbuk National University, Cheongju, Korea.
 SOURCE: MOLECULES AND CELLS, (1999 Oct 31) 9 (5) 560-3.
 Journal code: CRQ; 9610936. ISSN: 1016-8478.
 PUB. COUNTRY: KOREA (SOUTH)
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200002
 ENTRY DATE: Entered STN: 20000218
 Last Updated on STN: 20000218
 Entered Medline: 20000210

AB The TEL/AML1 ***fusion*** gene occurs in childhood B-cell acute lymphoblastic leukemia (ALL) as a result of the translocation of human chromosome 12;21. Using reporter gene assays, we have functionally characterized TEL, AML1 and TEL/AML1 ***fusion*** proteins in the regulation of the human CR1 gene. Analysis of transcription ***activities*** showed that AML1 ***increased*** the CR1 ***promoter*** ***activity*** and that TEL repressed the basal ***activity*** of the ***promoter***. ***Increased*** ***activities*** of the CR1 ***promoter*** by AML1 protein were reduced by the TEL protein in a concentration-dependent manner. When TEL/AML1 and AML1 proteins are present in cells at the same time, the TEL/AML1 protein inhibits the transactivation activities of AML1 protein on the human CR1 promoter even though TEL/AML1 retains the transactivation domain of AML1. A ***mutation*** analysis of the human CR1 ***promoter*** revealed that the binding sites for TEL and AML1 are necessary for the action of TEL and TEL/AML1, respectively. Thus, production of the TEL/AML1 protein by translocation of human chromosome 12;21 may contribute to leukemogenesis by the specific inhibition of AML1-dependent activation of myeloid promoters.

L18 ANSWER 13 OF 43 MEDLINE
 ACCESSION NUMBER: 2000062176 MEDLINE
 DOCUMENT NUMBER: 20062176 PubMed ID: 10597040
 TITLE: Role of -35 sequence and its cooperativity with vir-box for the expression of virE gene.
 AUTHOR: Han S S; Jeon G A; Sim W S
 CORPORATE SOURCE: Department of Biology, Korea University, Seoul.
 SOURCE: MOLECULES AND CELLS, (1999 Oct 31) 9 (5) 510-6.
 Journal code: CRQ; 9610936. ISSN: 1016-8478.
 PUB. COUNTRY: KOREA (SOUTH)
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200002
 ENTRY DATE: Entered STN: 20000218
 Last Updated on STN: 20000218
 Entered Medline: 20000210

AB To elucidate the role of the -35 sequence and its cooperativity with vir box in the expression of the virE gene, various mutants were constructed by either site-directed mutation or deletional ***mutation*** of the virE ***promoter***. The expression level of pHBV, a mutant where its putative -35 sequences (CCGAGT) have been substituted with the consensus -35 sequences of the Escherichia coli promoter (TTGACA), was increased by 386%. pECHV, containing the conserved -35 sequence but lacking the vir box and the 5'-half of the imperfect dyad symmetry region (DSR) showed an ***increase*** of 286% in its ***promoter*** ***activity***. pESHV, containing the conserved -35 sequence but lacking the complete 5'-upstream region from the mid-region of imperfect DSR, exhibited 244%

of

the native virE promoter activity. pHBV, containing the conserved -35 sequence but destroying the vir box, was constructed by substitution of A, C, T at the positions -62, -63, and -65 on the vir-box to T, A, C, respectively. These ***mutations*** ***increased***

promoter ***activities*** by 319%. On the other hand, when

the

vir box was mutated from imperfect DSR to almost perfect DSR with T to

A

and G to T substitutions at -60 and -61 positions of the virE promoter containing the conserved -35 sequence (pHBNA), a higher activity of 671%

was observed. These results demonstrate that when the putative -35 sequence of virE promoter is replaced with the consensus -35 sequence, the

virE gene can be expressed independently without the binding of VirG protein to the vir-box and/or the induction of acetosyringone. Moreover, the presence of an almost perfect dyad symmetry of the vir-box can increase the expression of virE synergistically with the consensus -35 sequence.

L18 ANSWER 14 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS DUPLICATE 6

ACCESSION NUMBER: 1999:432216 BIOSIS
 DOCUMENT NUMBER: PREV199900432216
 TITLE: Lysophosphatidylcholine phosphorylates CREB and activates

the jun2TRE site of c-jun promoter in vascular endothelial cells.

AUTHOR(S): Ueno, Yasushi; Kume, Noriaki (1); Miyamoto, Susumu; Morimoto, Masahumi; Kataoka, Hiroharu; Ochi, Hiroshi; Nishi, Eiichiro; Moriwaki, Hideaki; Minami, Manabu; Hashimoto, Nobuo; Kita, Toru

CORPORATE SOURCE: (1) Department of Geriatric Medicine, Graduate School of

Medicine, Kyoto University, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto, 606-8507 Japan

SOURCE: FEBS Letters, (Aug. 27, 1999) Vol. 457, No. 2, pp. 241-245.

ISSN: 0014-5793.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Lysophosphatidylcholine (lyso-PC), a polar phospholipid increased in atherogenic lipoproteins and atherosclerotic lesions, has been shown to induce transcription of a variety of endothelial genes relevant to atherogenesis. Lyso-PC has been shown to activate c-jun N-terminal kinase

(JNK) and activator protein 1 (AP-1) and thereby stimulate transcription of the c-jun gene. Here we provide evidence that lyso-PC can phosphorylate

cyclic AMP responsive element binding protein (CREB) and thereby activate

the jun2 12-O-tetradecanoylphorbol 13-acetate response element (jun2TRE)

site of the c-jun promoter, which appears to be the major molecular mechanism involved in lyso-PC-induced c-jun gene expression in cultured bovine aortic endothelial cells (BAEC). Transient transfection of BAEC with a 1.6-kbp c-jun promoter and luciferase reporter ***fusion*** gene resulted in a 12.9-fold increase in luciferase activity by lyso-PC treatment. Serial deletion ***mutation*** in c-jun ***promoter*** and luciferase reporter gene assay revealed that the 5' promoter region between nucleotide numbers -268 and -127, which contains a jun2TRE binding

sequence, was most crucial for lyso-PC-induced transcription. The 5' promoter region between -76 and -27, which contains an AP-1 site, also affected lyso-PC-induced transcription of the c-jun gene. Point mutation in the jun2TRE site reduced lyso-PC-induced transcription of the c-jun promoter-luciferase ***fusion*** gene by a 70.3% decrease in c-jun ***promoter*** ***activity***. Electrophoretic mobility shift assays showed ***increased*** binding of 32P-labeled oligonucleotides with jun2TRE in nuclear extracts isolated from lyso-PC-treated BAEC, which

was abolished or supershifted by anti-CREB antibody. Immunoblotting with anti-phosphorylated CREB antibody showed rapid phosphorylation of this protein after lyso-PC treatment. These results indicate that lyso-PC phosphorylates CREB, which was then bound to the jun2TRE site of the c-jun

promoter and activated transcription. Activation of *jun2TRE* may play a key role in the transcriptional activation of *c-jun* as well as other endothelial genes depending upon these transcription factors.

L18 ANSWER 15 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
DUPLICATE 7

ACCESSION NUMBER: 1999:342302 BIOSIS
DOCUMENT NUMBER: PREV199900342302

TITLE: ***Mutational*** analysis of the ***promoter*** region of the *porA* gene of *Neisseria meningitidis*.

AUTHOR(S): Sawaya, R.; Arhin, F. F.; Moreau, F.; Coulton, J. W.; Mills, E. L. (1)

CORPORATE SOURCE: (1) Department of Microbiology and Immunology, McGill University, 3775 University Street, Montreal, PQ, H3A 2B4 Canada

SOURCE: Gene (Amsterdam), (June 11, 1999) Vol. 233, No. 1-2, pp.

49-57.

ISSN: 0378-1119.

DOCUMENT TYPE: Article

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The *porA* gene encodes the class 1 outer membrane protein (OMP1) in *Neisseria meningitidis* and is under transcriptional control. Promoter regions of *porA* from different clinical isolates were sequenced and were found to differ in the number of guanosine residues in a poly(G) track located upstream of the -10 region. Isolates that did not express OMP1

had up to nine G residues in the poly(G) track or an adenosine residue within this poly(G) track. Using beta-galactosidase as a reporter gene, the transcriptional activities of the promoter regions of the *porA* gene from three strains, two of which do not express OMP1, were assayed in both *Escherichia coli* and *N. meningitidis*. Mutations in the poly(G) track were created by site-directed mutagenesis and promoter ***fusions*** were further analyzed in *E. coli* and *N. meningitidis*. The number of nucleotides in the poly(G) track influenced promoter activity: reduction of a poly(G) track of 12 nt by one and by two guanosine residues reduced promoter activity. Within the poly(G) track, replacement of an adenosine residue by a guanosine residue ***increased*** the ***promoter*** ***activity***; replacement of a guanosine residue by an adenosine residue decreased the activity. The similar transcriptional activities for the ***mutated*** ***promoters*** in *E. coli* and *N. meningitidis* are compatible with similar control mechanisms for transcriptional control in both organisms.

L18 ANSWER 16 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
DUPLICATE 8

ACCESSION NUMBER: 1998:165368 BIOSIS

DOCUMENT NUMBER: PREV199800165368

TITLE: A CACCC box in the proximal exon 2 promoter of the rat insulin-like growth factor 1 gene is required for basal promoter activity.

AUTHOR(S): Wang, Xia; Talamantez, Jose L.; Adamo, Martin L. (1)
CORPORATE SOURCE: (1) Dep. Biochem., Univ. Texas Health Sci. Cent., 7703

Floyd Curl Dr., San Antonio, TX 78284-7760 USA
SOURCE: Endocrinology, (March, 1998) Vol. 139, No. 3, pp. 1054-1066.

ISSN: 0013-7227.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The insulin-like growth factor 1 gene is transcribed from two promoter regions, resulting in alternative first exons in insulin-like growth factor 1 messenger RNAs. A previous study showed that the sequence from

-73 to +44 (where +1 is the first nucleotide in the exon 2 transcription initiation cluster) contained an active exon 2 promoter, and that sequences between -73 and -36 were required for promoter activity. In the current study, the roles of two putative cis-acting elements within the -73 to +44 region in basal exon 2 promoter activity were evaluated using mutagenesis and nuclear protein-DNA binding assays. Mutation of the CCCCACCC sequence at position -53 to GAAATCCC resulted in a complete loss

of promoter activity in transient transfection assays in GH3, OVCAR-3, C6, and Chinese hamster ovary (CHO) cells. A -73/+24 exon 2

promoter-luciferase construct had partial ***promoter*** activity.

Mutation of a putative initiator motif surrounding the major exon 2 start site did not alter the activity of this construct. In electrophoretic mobility shift assays, a 32P-labeled oligomer extending from -73 to +44 in the exon 2 promoter was specifically bound by GH3

cell nuclear extracts. A 32P-labeled oligomer which extended from -63 to -37

in the exon 2 promoter was specifically bound by GH, and OVCAR-3 cell nuclear extracts. These unlabeled oligomers inhibited the binding of a labeled -236/+44 exon 2 promoter fragment to OVCAR-3 nuclear extracts.

Mutation of the CCCCACCC sequence prevented the unlabeled -73/+44 oligomer from

inhibiting the binding of the -236/+44 fragment. An unlabeled oligomer containing a consensus activating protein-2 (AP-2)-binding site inhibited labeled -236/+44, -73/+44, and -63/-37 exon 2 promoter binding with a

much lower affinity than did the respective unlabeled oligomers. Purified AP-2 protein did not bind to the exon 2 promoter fragment, nor did anti-AP-2 antibody alter the binding. Cotransfection of AP-2 expression vector did not significantly ***increase*** exon 2 ***promoter***

activity. On the other hand, an oligomer containing a consensus Sp1-binding site inhibited labeled -63/-37 exon 2 promoter binding by GH, cell nuclear extracts with an affinity similar to that of the unlabeled -63/-37 oligomer. A mutation in the Sp1-binding site in this same oligomer resulted in a complete loss of binding affinity. Purified Sp1 bound to the -63/-37 exon 2 promoter oligomer. Addition of polyclonal antibody to Sp1 resulted in a partial supershift of the complex formed between GH, cell and OVCAR-3 cell nuclear extracts and the labeled -63/-37 oligomer. However, in *Drosophila Schneider* cells, which are an experimental model for studying the ability of Sp1 to activate transcription, the -73/+44 exon 2 promoter construct was not stimulated by cotransfection with an

Sp1 expression plasmid. UV cross- ***linking*** studies indicated that proteins of approximate molecular mass 125, 76, 47, and 38 kDa are bound

to the proximal (-236/+44) exon 2 promoter region. It is concluded that the CCCCACCC sequence at -53 is required for exon 2 promoter activity. Moreover, specific binding of nuclear proteins to the proximal exon 2 promoter region requires the CCCCACCC sequence. Sequences downstream of

the exon 2 initiation site from +24 to +44 are required for full promoter activity. However, the putative initiator surrounding the major transcription start site at +1 does not appear to be important for the strength of the proximal promoter. The CCCCACCC sequence at -53 appears

to be a CACCC box, which binds zinc finger transcription factors of the Kruppel family such as Sp1, although protein factors in addition to Sp1 are required to activate exon 2 transcription through the -73/+44 proximal promoter region.

L18 ANSWER 17 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
DUPLICATE 9

ACCESSION NUMBER: 1998:391099 BIOSIS

DOCUMENT NUMBER: PREV199800391099

TITLE: Pathways for transcriptional activation of a glutathione-dependent formaldehyde dehydrogenase gene.

AUTHOR(S): Barber, Robert D.; Donohue, Timothy J. (1)

CORPORATE SOURCE: (1) Dep. Bacteriol., Univ. Wisconsin-Madison, Madison, WI 53706 USA

SOURCE: Journal of Molecular Biology, (July 31, 1998) Vol. 280, No.

5, pp. 775-784.

ISSN: 0022-2836.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The widespread occurrence of glutathione-dependent formaldehyde dehydrogenases (GSH-FDH) suggests that this enzyme serves a conserved function in preventing the cytogenetic and potentially lethal interaction of formaldehyde with nucleic acids, proteins and other cell constituents. Despite this potential role of GSH-FDH, little is known about how its expression is regulated. Here, we identify metabolic and genetic signals that activate transcription of a GSH-FDH gene (*adhI*) in the bacterium *Rhodobacter sphaeroides*. ***Activity*** of the *adhI* ***promoter*** is ***increased*** by both exogenous formaldehyde and metabolic

sources of this toxin. Elevated adh1 promoter activity in
 DELTAGSH-FDH
 mutants implicates formaldehyde or the glutathione adduct that serves as a
 GSH-FDH substrate, S-hydroxymethylglutathione, as a transcriptional
 effector. From studying adh1 expression in different host mutants, we find
 that the photosynthetic response regulator PrrA and the trans-acting spd-7
 mutation increase function of this ***promoter***. The
 behavior of a nested set of adh1::lacZ ***fusions*** indicates that
 activation by formaldehyde, PrrA and spd-7 requires only sequences 55 bp
 upstream of the start of transcription. A working model is presented to
 explain how GSH-FDH expression responds to formaldehyde and global
 signals
 generated from the reduced pyridine nucleotide produced by the activity of
 this enzyme.

L18 ANSWER 18 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 10
 ACCESSION NUMBER: 1998:164028 BIOSIS
 DOCUMENT NUMBER: PREV199800164028
 TITLE: ***Mutations*** in the ***promoter*** of adenylyl
 cyclase (AC)-III gene, overexpression of AC-III mRNA, and
 enhanced cAMP generation in islets from the spontaneously
 diabetic GK rat model of type 2 diabetes.
 AUTHOR(S): Abdel-Halim, Samy M. (1); Gueniff, Amel; He, Bing;
 Yang, Bei; Mustafa, Maha; Hojeberg, Bo; Hillert, Jan; Bakhiet,
 Moiz; Efendic, Suda
 CORPORATE SOURCE: (1) Endocrine Diabetes Unit, Dep. Mol. Med.,
 Karolinska Hosp., L1-02, S-171 76 Stockholm Sweden
 SOURCE: Diabetes, (March, 1998) Vol. 47, No. 3, pp. 498-504.
 ISSN: 0012-1797.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 AB Glucose-induced insulin release is decreased in the spontaneously
 diabetic

GK rat, a nonobese rodent model of type 2 diabetes. Forskolin restores the
 impaired insulin release in both the isolated perfused pancreas and
 isolated islets from these rats (Abdel-Halim et al., Diabetes 45:934-940,
 1996). We demonstrate here that the insulinotropic effect of forskolin in
 the GK rat is due to increased generation of cAMP and that it is
 associated with overexpression of adenylyl cyclase (AC)-III mRNA and
 gene
 mutations. The AC-III mRNA overexpression was demonstrated by in situ
 hybridization using oligonucleotide probes binding to different regions of
 the rat AC-III mRNA. It was associated with the presence of two point
 mutations identified at positions -28 bp (A fwdarw G) and -358 bp (A
 fwdarw C) of the promoter region of the AC-III gene and was
 demonstrable in
 both GK rat islets and peripheral blood cells. Transfection of COS cells
 with a luciferase reporter gene system revealed up to 25-fold
 increased ***promoter*** ***activity*** of GK AC-III
 promoter when compared with normal rat promoter (P <
 0.0001). In
 conclusion, forskolin restores the impaired insulin release in islets of
 the GK rat through enhanced cAMP generation. This is ***linked*** to
 overexpression of AC-III mRNA in GK islets due to two functional point
 mutations in the ***promoter*** region of the AC-III gene.

L18 ANSWER 19 OF 43 MEDLINE
 ACCESSION NUMBER: 1999015774 MEDLINE
 DOCUMENT NUMBER: 99015774 PubMed ID: 9801155
 TITLE: GATA-3 represses gp91phox gene expression in
 eosinophil-committed HL-60-C15 cells.
 AUTHOR: Sadat M A; Kumatori A; Suzuki S; Yamaguchi Y; Tsuji
 Y;
 Nakamura M
 CORPORATE SOURCE: Department of Pediatrics, Nagasaki University
 School of
 Medicine, Sakamoto, Japan.. fl106@cc.nagasaki-u.ac.jp
 SOURCE: FEBS LETTERS, (1998 Oct 9) 436 (3) 390-4.
 Journal code: EUH; 0155157. ISSN: 0014-5793.
 PUB. COUNTRY: Netherlands
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199811
 ENTRY DATE: Entered STN: 19990106

Last Updated on STN: 19990106
 Entered Medline: 19981123
 AB To study the regulatory mechanism of gp91phox gene expression in
 eosinophils, we transiently transfected eosinophil-committed HL-60-C15
 cells with gp91phox promoter constructs, and identified a negative
 element
 from bp -267 to -246 of the gp91phox gene, the deletion of which caused
 an
 83% ***increase*** in ***promoter*** ***activity***.
 Electrophoresis mobility shift assays demonstrated GATA-3 binds to the
 GATA consensus site from bp -256 to -250. An 81% increment in
 promoter activity was obtained when a ***mutation*** was
 introduced in the GATA-3 binding site of the bp -267 to +12 construct,
 which is comparable to that of the bp -245 to +12 construct. We therefore
 conclude that GATA-3 specifically binding to the GATA site negatively
 regulates the expression of the gene in HL-60-C15 cells.

L18 ANSWER 20 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 11
 ACCESSION NUMBER: 1998:411323 BIOSIS
 DOCUMENT NUMBER: PREV199800411323
 TITLE: Possible role of c-Jun in transcription of the mouse renin
 gene.
 AUTHOR(S): Tamura, Kouichi (1); Umemura, Satoshi; Nyui, Nobuo;
 Yabana, Machiko; Toya, Yoshiyuki; Fukamizu, Akiyoshi; Murakami,
 Kazuo; Ishii, Masao
 CORPORATE SOURCE: (1) Dep. Internal Med. II, Yokohama City Univ.
 Sch. Med.,
 3-9 Fukuura, Kanazawa-Ku, Yokohama 236 Japan
 SOURCE: Kidney International, (Aug., 1998) Vol. 54, No. 2, pp.
 382-393.
 ISSN: 0085-2538.
 DOCUMENT TYPE: Article
 LANGUAGE: English

AB Background. Renin is a rate-limiting enzyme for activity of the
 circulating renin-angiotensin system (RAS) and expression of the renin
 gene is regulated by a variety of stimuli. In this study, we examined a
 possible role of c-Jun in the transcription of renin gene. Methods. The
 renin promoter, chloramphenicol acetyltransferase (CAT), ***fusion***
 genes with or without c-Jun expression vector (pSV-c-Jun) were
 transfected
 into human embryonic kidney (HEK) cells, and the effects of c-Jun were
 examined by deletion and mutation analyses of CAT assay and by in vitro
 transcription-primer extension assay. We also examined the effects of
 c-Jun on DNA-binding activity to the renin promoter by electrophoretic
 mobility shift assay (EMSA). Furthermore, we examined the effects of
 c-Jun
 on transcription of the renin gene in enriched juxtaglomerular (JG) cells
 by cotransfection with pSVc-Jun and by treatment with antisense c-jun
 oligodeoxynucleotides. Results. ***Promoter*** ***activity*** of
 the renin gene was ***increased*** by c-Jun overexpression in HEK
 cells, and the proximal promoter region from -47 to +16 was sufficient for
 transcriptional activation by c-Jun. Although mutation of activator
 protein-1 (AP-1) element-like sequences in the proximal promoter did not
 affect c-Jun-mediated stimulation, ***mutation*** of the core
 promoter including the TATA box inhibited c-Jun-mediated
 transcription. The results of EMSA showed that c-Jun overexpression
 produced a binding of nuclear factor, which was HEK cell-specific and
 distinct from TATA box-binding protein and AP-1 family transcription
 factor, to the renin core promoter region (RC element) from -36 to -20.
 The overexpression of c-Jun activated the renin promoter in
 renin-expressing JG cells, and antisense c-jun decreased the activity of
 renin promoter and expression of renin mRNA in JG cells. Conclusions.
 These results indicate that the RC element plays a role in c-Jun-mediated
 transcriptional regulation of the renin gene in HEK cells, and suggest
 that c-Jun participates in the regulation of renin gene expression in JG
 cells of the kidney.

L18 ANSWER 21 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 12
 ACCESSION NUMBER: 1998:495726 BIOSIS
 DOCUMENT NUMBER: PREV199800495726
 TITLE: A CT repeat in the promoter of the chicken malic enzyme
 gene is essential for function at an alternative
 transcription start site.
 AUTHOR(S): Xu, Gang; Goodridge, Alan G. (1)
 CORPORATE SOURCE: (1) Coll. Biol. Sci., Ohio State Univ., 484 W.

12th Ave.,
Columbus, OH 43210 USA
SOURCE: Archives of Biochemistry and Biophysics, (Oct. 1, 1998)
Vol. 358, No. 1, pp. 83-91.
ISSN: 0003-9861.

DOCUMENT TYPE: Article

LANGUAGE: English

AB CT repeats are abundant in eukaryotic genomes and have been implicated in

a number of biological events. The promoter of the chicken malic enzyme gene contains a long polypyrimidine/polypurine tract that includes seven tandem CTs. This CT repeat region together with 14 immediately downstream

nucleotides functions as an active alternative promoter when

linked to a reporter gene and may direct transcription initiation at a cluster of minor sites in the endogenous gene (G. Xu and A. G. Goodridge (1996) J. Biol. Chem. 271, 16008-16019). In the sequence required for promoter activity, -105 to -83 bp, there are two purines; only the A at -83 bp influences ***promoter*** activity.

Mutation of different four-nucleotide stretches of the CT repeats to purines decreased ***promoter*** ***activity*** as a function of the ***increase*** in GC content. Increasing the number of CT repeats by changing pyrimidines downstream of (CT)7 to CTs ***increased*** ***promoter*** ***activity***. These sequences and other regions showed moderate sensitivity to S1 nuclease in supercoiled plasmids, suggesting the presence of non-B-DNA structures. Increasing the length of the CT repeats should increase the propensity to adopt non-B-DNA structures such as triplexes. Constructs with 10, 15, or 22 repeats had increased expression relative to wild type. Thus, the ability of CT repeats to form non-B-DNA structures may be functionally important.

L18 ANSWER 22 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
DUPLICATE 13

ACCESSION NUMBER: 1997:391409 BIOSIS

DOCUMENT NUMBER: PREV199799690612

TITLE: The WT1 protein is a negative regulator of the normal bcl-2 allele in t(14;18) lymphomas.

AUTHOR(S): Heckman, Caroline; Mochon, Evonne; Arcinas, Magdalena;

Boxer, Linda M. (1)

CORPORATE SOURCE: (1) Div. Hematol., S-161, Stanford Univ. Sch. Medicine,

Stanford, CA 94305-5112 USA

SOURCE: Journal of Biological Chemistry, (1997) Vol. 272, No. 31,
pp. 19609-19614.
ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The translocated and normal bcl-2 alleles in the DHL-4 cell line with the t(14;18) translocation were separated by pulsed field electrophoresis. An in vivo footprint over a potential WT1 binding site in the bcl-2 5'-flanking sequence was identified on the normal silent allele. Electrophoretic mobility shift assays with the bcl-2 WT1 site

demonstrated a single specific complex. UV cross- ***linking*** and Western analysis revealed that this gel shift complex contained WT1 protein. Deletion or mutation of the WT1 site resulted in an ***increase*** in

activity of the bcl-2 ***promoter*** in DHL-4 cells. Cotransfection with a 3:1 ratio of a WT1 expression vector to the bcl-2 promoter construct led to a 3.0-fold repression of the bcl-2 promoter. Cotransfection with a WT1 expression vector and the bcl-2

promoter with the ***mutated*** WT1 site resulted in only 1.2-fold repression. We conclude that the WT1 site functions as a negative regulatory site for the normal silent bcl-2 allele in t(14;18) lymphomas. The WT1 site is not occupied on the translocated bcl-2 allele.

L18 ANSWER 23 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
DUPLICATE 14

ACCESSION NUMBER: 1997:456861 BIOSIS

DOCUMENT NUMBER: PREV199799756064

TITLE: Src-mediated activation of the human neurotensin/neuromedin N promoter.

AUTHOR(S): Banker, Nitesh A.; Hellmich, Mark R.; Kim, Hong Jin; Townsend, Courtney M., Jr.; Evers, B. Mark (1)

CORPORATE SOURCE: (1) Dep. Surg., Univ. Texas Med. Branch, 301 University

Blvd., Galveston, TX 77555-0533 USA

SOURCE: Surgery (St Louis), (1997) Vol. 122, No. 2, pp. 180-186.
ISSN: 0039-6060.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Background. Expression of the gene encoding the neurotensin/neuromedin N

(NT/N) is developmentally regulated in the gut in a distinctive temporal and spatial fashion. Src kinase, a nonreceptor tyrosine kinase, has been implicated in the growth and differentiation of various tissues; its role in gut differentiation is not known. The purpose of this study was to determine whether the Src signaling pathway plays a role in the activation of the human NT/N promoter. Methods. Caco-2 cells, a human colon cancer cell line that can differentiate to a small bowel phenotype, were transiently transfected with human NT/N promoter fragments

linked to luciferase and various amounts of Src expression plasmids or dominant negative Raf; luciferase and beta-galactosidase activities were measured after 48 hours. Results. Cotransfection of Src resulted in an approximate eight-fold ***increase*** of NT/N ***promoter*** ***activity***; ***mutation*** of a proximal activating protein-1/cyclic adenosine monophosphate responsive element site resulted in a dramatic decrease of Src-mediated NT/N induction. Cotransfection with a dominant negative Raf

plasmid partially blocked Src-mediated NT/N activation. Conclusions. Src ***increases*** NT/N ***promoter*** ***activity*** in Caco-2 cell acting, in part, through a proximal AP-1/CRE promoter element. In addition, Src regulation of the NT/N promoter appears to be mediated in the gut.

L18 ANSWER 24 OF 43 MEDLINE

ACCESSION NUMBER: 96347543 MEDLINE

DOCUMENT NUMBER: 96347543 PubMed ID: 8756632

TITLE: The nuclear factor YY1 suppresses the human gamma interferon promoter through two mechanisms: inhibition of AP1 binding and activation of a silencer element.

AUTHOR: Ye J; Cipitelli M; Dorman L; Ortaldo J R; Young H A
CORPORATE SOURCE: Laboratory of Experimental Immunology, Division of Basic

Sciences, National Cancer Institute-Frederick Cancer Research and Developmental Center, Maryland 21702-1201, USA.

SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1996 Sep) 16 (9) 4744-53.

Journal code: NGY; 8109087. ISSN: 0270-7306.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199609

ENTRY DATE: Entered STN: 19961008

Last Updated on STN: 19970203

Entered Medline: 19960926

AB Our group has previously reported that the nuclear factor Yin-Yang 1 (YY1), a ubiquitous DNA-binding protein, is able to interact with a silencer element (BE) in the gamma interferon (IFN-gamma) promoter region.

In this study, we demonstrated that YY1 can directly inhibit the activity of the IFN-gamma promoter by interacting with multiple sites in the promoter. In cotransfection assays, a YY1 expression vector significantly inhibited IFN-gamma ***promoter*** activity. ***Mutation*** of the

YY1 binding site in the native IFN-gamma ***promoter*** was associated with an ***increase*** in the IFN-gamma ***promoter*** ***activity***. Analysis of the DNA sequences of the IFN-gamma promoter

revealed a second functional YY1 binding site (BED) that overlaps with an AP1 binding site. In this element, AP1 enhancer activity was suppressed by

YY1. Since the nuclear level of YY1 does not change upon cell activation, our data support a model that the nuclear factor YY1 acts to suppress basal IFN-gamma transcription by interacting with the promoter at multiple

DNA binding sites. This repression can occur through two mechanisms: (i) cooperation with an as-yet-unidentified AP2-like repressor protein and (ii) competition for DNA binding with the transactivating factor AP1.

L18 ANSWER 25 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS

DUPLICATE 15

ACCESSION NUMBER: 1996:132571 BIOSIS

DOCUMENT NUMBER: PREV199698704706

TITLE: Interaction of ethanol with inducers of glucose-regulated stress proteins: Ethanol potentiates inducers of grp78 transcription.

AUTHOR(S): Hsieh, Kwei-Perng; Wilke, Nobert; Harris, Adron; Miles,

Michael F. (1)

CORPORATE SOURCE: (1) Bldg. 1 Rm 101, San Francisco Gen. Hosp., 1001 Potrero

Ave., San Francisco, CA 94110 USA

SOURCE: Journal of Biological Chemistry, (1996) Vol. 271, No. 5, pp. 2709-2716.

ISSN: 0021-9258.

DOCUMENT TYPE: Article

LANGUAGE: English

AB GRP78, a molecular chaperone expressed in the endoplasmic reticulum, is a

"glucose-regulated protein" induced by stress responses that deplete glucose or intracisternal calcium or otherwise disrupt glycoprotein trafficking. Previously we showed that chronic ethanol exposure increases the expression of GRP78. To further understand the mechanism

underlying

ethanol regulation of GRP78 expression, we studied the interaction between

ethanol and classical modulators of GRP78 expression in NG108-15 neuroblastoma times glioma cells. We found that, in addition to increasing basal levels of GRP78 mRNA ("induction"), ethanol produced greater than additive increases in the induction of GRP78 mRNA by the "classical"

GRP

inducers A23187, brefeldin A, and thapsigargin ("potentiation"). Both the ethanol induction and potentiation responses modulated grp78 gene transcription as determined by stable transfection analyses with the rat grp78 promoter. Ethanol potentiated the action of all classical inducers of grp78 transcription that were studied. In contrast, co-treatment with the classical GRP inducers thapsigargin and tunicamycin produced only simple additive ***increases*** in grp78 ***promoter***

activity. Transient transfection studies with deletion mutants of the rat grp 78 promoter showed that cis-acting promoter sequences

required

for ethanol induction differ from those mediating responses to classical GRP inducers. Furthermore, ***linker***-scanning ***mutations*** of the grp78 ***promoter*** suggested that the ethanol potentiation response required a cis-acting promoter element different from those involved in induction by ethanol or classical inducing agents. While the ethanol induction response required 16-24 h to be detectable, ethanol potentiation of thapsigargin occurred within 6 h. The potentiation response also decayed rapidly after ethanol removal. In addition, the protein kinase A inhibitor R-p-cAMPS and protein phosphatase inhibitor okadaic acid both increased ethanol potentiation of thapsigargin while S-p-cAMPS, an activator of protein kinase A, decreased ethanol potentiation. Taken together, our findings suggest two mechanisms by

which

ethanol regulates grp78 transcription, both differing from the action of classical GRP inducers such as thapsigargin. One mechanism

(potentiation)

involves a protein phosphorylation cascade and potentiates the action of classical GRP inducers. In contrast, GRP78 induction by ethanol involves promoter sequences and a mechanistic pathway separate from that of the ethanol potentiation response or classical GRP78 inducers. These studies show that ethanol produces a novel and complex regulation of grp78 transcription which could be of particular importance during neuronal exposure to GRP-inducing stressors as might occur with central nervous system injury.

L18 ANSWER 26 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS

DUPLICATE 16

ACCESSION NUMBER: 1996:575367 BIOSIS

DOCUMENT NUMBER: PREV199799290048

TITLE: Transcriptional activation of the GLUT2 gene by the IPF-1/STF-1/IDX-1 homeobox factor.

AUTHOR(S): Waeber, Gerard (1); Thompson, Nancy; Nicod, Pascal;

Bonny,

Christophe

CORPORATE SOURCE: (1) Dep. Internal Med., B/19-135, Centre Hospitalier Univ.

Vaudois, CHUV-1011 Lausanne Switzerland

SOURCE: Molecular Endocrinology, (1996) Vol. 10, No. 11, pp. 1327-1334.

ISSN: 0888-8809.

DOCUMENT TYPE: Article

LANGUAGE: English

AB The homeodomain protein PDX-1, referred as IPF-1/STF-1/IDX-1, is a transcriptional factor that plays a critical role in the control of several genes expressed in the pancreatic islet. PDX-1 gene expression

has been previously shown to be reduced in cultured beta-cell lines chronically exposed to high glucose concentrations. As the glucose transporter type 2 (GLUT2) gene expression is selectively decreased in the

beta-pancreatic cells of experimental models of diabetes, we postulated that the loss of GLUT2 gene expression in the pancreatic islets of diabetic animals may be due to the loss of PDX-1 transacting function on the GLUT2 gene. We, therefore, investigated the potential role of PDX-1

in the transcriptional control of GLUT2. We have identified a repeat of a TAAT motif (5'-TAATA-ATAACA-3') conserved in the sequence of the human and

murine GLUT2 promoters. Recombinant PDX-1 binds to this GLUT2TAAT motif in

electrophoretic mobility shift experiments. PDX-1 antiserum detects the formation of the complex of PDX-1 with the GLUT2TAAT motif in nuclear

extracts from the pancreatic insulin-secreting cell line, beta-TC3. The GLUT2TAAT motif was ***mutated*** in the murine GLUT2

promoter

(-1308/+49 bp) ***linked*** to a luciferase reporter gene and transfected into beta-TC3 cells. ***Compared*** with the transcriptional ***activity*** of the wild type ***promoter***, that of the ***mutated*** ***promoter*** decreases by 41%.

Multiple copies of the GLUT2TAAT motif were ligated 5' to a heterologous

promoter and transfected into a PDX-1-expressing cell line (beta-TC3) and

into cell lines lacking the homeobox factor (InR1-G9 and JEG-3). The GLUT2TAAT motif mediates the activation of the heterologous promoter

in the PDX-1-expressing cell line but not in InR1-G9 or JEG-3 cell lines.

Furthermore, cotransfection in a PDX-1-deficient cell line with the expression vector encoding PDX-1 transactivates specifically the heterologous promoter containing the multimerized GLUT2TAAT motif.

These

data demonstrate that the murine GLUT2 promoter is controlled by the

PDX-1

homeobox factor through the identified GLUT2TAAT motif.

L18 ANSWER 27 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS

DUPLICATE 17

ACCESSION NUMBER: 1996:243331 BIOSIS

DOCUMENT NUMBER: PREV199698791460

TITLE: Requirement of a CCGAC cis-acting element for cold induction of the BN115 gene from winter Brassica napus.

AUTHOR(S): Jiang, Chao; Iu, Betty; Singh, Jas (1)

CORPORATE SOURCE: (1) Plant Res. Centre, Agriculture Canada, Central

Experimental Farm, Ottawa, ON K1A 0C6 Canada

SOURCE: Plant Molecular Biology, (1996) Vol. 30, No. 3, pp. 679-684.

ISSN: 0167-4412.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Mutation of the core pentamer, CCGAC, of two putative low temperature

responsive elements (LTREs) in the 5'-proximal region of the winter Brassica napus cold-induced gene BN115 was carried out. Analyses of transient expression of the resultant ***mutated*** BN115

promoter -GUS ***fusions*** revealed the loss of low-temperature regulation by the promoter. This indicates that the CCGAC

sequence is critical to the low-temperature response in the BN115 gene. In

contrast, mutation of two G-boxes, CACGTG, staggered between the LTREs in

the same region of the promoter did not alter cold-inducible gene expression. Replacement of a possible enhancer region of the BN115 promoter with the enhancer from the CaMV 35S ***promoter*** resulted

in a several-fold ***increase*** in low temperature-induced GUS ***activity***

L18 ANSWER 28 OF 43 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 18

ACCESSION NUMBER: 95314759 EMBASE

DOCUMENT NUMBER: 1995314759

TITLE: Two ***mutations*** in the ***promoter*** region of the human protein C gene both cause type I protein C deficiency by disruption of two HNF-3 binding sites.

AUTHOR: Spek C.A.; Greengard J.S.; Griffin J.H.; Bertina R.M.; Reitsma P.H.

CORPORATE SOURCE: Dept. of Hematology, Hemostasis and Thrombosis Res. Ctr., University Hospital, P. O. Box 9600, 2300 CR Leiden, Netherlands

SOURCE: Journal of Biological Chemistry, (1995) 270/41 (24216-24221).

ISSN: 0021-9258 CODEN: JBCHA3

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 025 Hematology

029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB Protein C is a vitamin K-dependent zymogen of a serine protease that inhibits blood coagulation by the proteolytic inactivation of factors Va and VIIIa. Individuals affected with protein C deficiency are at risk for thrombosis. Genetic analyses of affected individuals, to determine the cause of the protein C deficiency, revealed a large variety of mutations in the protein C gene, including several in the promoter region of this gene. Comparison of the region around two of these mutations, A-32 .fwdarw. G and T-27 .fwdarw. A, with transcription factor consensus sequences suggested the presence of two overlapping and inversely oriented

HNF-3 binding sites. Direct evidence for the presence of the two HNF-3 binding sites in the protein C promoter was obtained using electrophoretic mobility shift assays and UV cross- ***linking*** experiments. These experiments revealed that HNF-3 can bind specifically to both putative HNF-3 sites in the wild-type protein C promoter. Due to the T-27 .fwdarw.

A mutation, one binding site is completely lost, while the other site still binds HNF-3, but with strongly reduced affinity. As a consequence of the A-32 .fwdarw. G ***mutation***, the protein C ***promoter*** loses all its HNF-3 binding capacity. Transient transfection experiments demonstrated that the binding of HNF-3 to the protein C promoter is of physiological significance. This followed from experiments in which the introduction of the A-32 .fwdarw. G or T-27 .fwdarw. A mutation resulted in a 4-5-fold reduced promoter activity in HepG2 cells. Furthermore, transactivation of the wild-type protein C promoter construct with HNF-3 shorted a 4-5-fold ***increased*** ***promoter*** ***activity*** in HepG2 cells. In HeLa cells, significant wild-type promoter activity was only observed after transactivation with HNF-3. When a promoter construct containing the T-27 .fwdarw. A mutation at position -27 was used, the transactivation potential of HNF-3 was 2-fold reduced in HepG2 cells, whereas in HeLa cells no transactivation was observed. With the promoter construct containing the A-32 .fwdarw. G mutation, no transactivation by HNF-3 was found either in HepG2 or in HeLa cells.

L18 ANSWER 29 OF 43 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 19

ACCESSION NUMBER: 95267354 EMBASE

DOCUMENT NUMBER: 1995267354

TITLE: MYB binding sites mediate negative regulation of c-MYB expression in T-cell lines.

AUTHOR: Guerra J.; Withers D.A.; Boxer L.M.

CORPORATE SOURCE: Division of Hematology, Stanford Univ. School of

Medicine, Stanford, CA 94305-5112, United States

SOURCE: Blood, (1995) 86/5 (1873-1880).

ISSN: 0006-4971 CODEN: BLOOAW

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 025 Hematology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB In hematopoietic cell development, the c-myb transcription factor plays an

important role. c-myb mRNA is expressed at high levels in immature proliferating cells and in leukemic cells. We have investigated the regulatory role of Myb protein binding to the human c-myb promoter.

Three

Myb binding sites have been described at approximately 600 bp upstream of

the cap site. By transient transfection assays in hematopoietic cell lines, we found that deletion of the previously defined most 5' Myb binding site had no effect on activity, whereas deletion of the region containing the remaining two Myb binding sites resulted in an increase in activity in both a T-cell line and a myeloid cell line. To specifically test the importance of these two Myb binding sites, the activity of three-point mutation constructs was measured. Mutation of either Myb binding site resulted in an ***increase*** in ***activity*** ***compared*** with the wild-type ***promoter*** in T cells. ***Mutation*** of both sites produced even higher activity.

Transfection

of the Myb site mutants into the myeloid cell line resulted in no change in activity compared with the wild type construct. Results from gel shift analysis, UV cross- ***linking***, and Western blots showed that both c-Myb and B-Myb bound to the Myb I and II sites. We conclude that the Myb

family proteins negatively regulate c-myb expression in T-cell lines in contrast to the positive regulation via these sites, which has been shown in fibroblasts. In addition, in a myeloid cell line, the Myb binding sites are nonfunctional.

L18 ANSWER 30 OF 43 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:970217 HCAPLUS

DOCUMENT NUMBER: 124:78358

TITLE: Developmental control of N-CAM expression by Hox and Pax gene products

AUTHOR(S): Edelman, Gerald M.; Jones, Frederick S.

CORPORATE SOURCE: Department of Neurobiology, Scripps Research

Institute, La Jolla, CA, 92037, USA

SOURCE: Philos. Trans. R. Soc. London, Ser. B (1995), Volume

Date 1995, 349(1329), 305-12

CODEN: PTRBAE; ISSN: 0080-4622

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A mounting body of evidence suggests that cell-cell adhesion mols. (CAMs)

play crit. roles in morphogenetic patterning. Perturbations of CAM binding can lead to altered tissue pattern and interruption of tissue interactions can lead to altered patterns of CAM expression. These observations focus attention on the factors responsible for the place-dependent expression of adhesion mols. such as N-CAM, the neural cell adhesion mol. Our recent expts. in vitro indicate that transcription factors encoded by Hox and Pax genes bind to specific DNA sequences in the

N-CAM promoter and activate that promoter. In particular, a region of the N-CAM promoter designated the homeodomain binding site (HBS) interacts

with a variety of different homeodomain proteins. A different region of the N-CAM promoter binds to the paired domain of Pax proteins. These transcription factors differentially regulate the N-CAM gene. Such in vitro studies suggest that the N-CAM gene may be an in vivo target for homeobox and Pax gene products. Recent expts. on transgenic mice

carrying normal and ***mutated*** segments of the N-CAM ***promoter*** ***linked*** to a lacZ reporter gene suggest that the N-CAM regulation

obsd. in vitro actually has counterparts in vivo. The significance of these observations is that they connect gene products capable of morpho-regulation (such as CAMs) to pattern-forming genes.

L18 ANSWER 31 OF 43 MEDLINE

ACCESSION NUMBER: 94268493 MEDLINE

DOCUMENT NUMBER: 94268493 PubMed ID: 8208240

TITLE: The positive and negative cis-acting elements for methanol regulation in the Pichia pastoris AOX2 gene.

AUTHOR: Ohi H; Miura M; Hiramatsu R; Ohmura T
SOURCE: MOLECULAR AND GENERAL GENETICS, (1994 Jun 3) 243 (5)

489-99.

Journal code: NGP; 0125036. ISSN: 0026-8925.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-X79871

ENTRY MONTH: 199407

ENTRY DATE: Entered STN: 19940721

Last Updated on STN: 19980206

Entered Medline: 19940712

AB The methylotrophic yeast *Pichia pastoris* has two alcohol oxidase genes, AOX1 and AOX2. The AOX2 gene is transcribed at a much lower level than the

AOX1 gene. Apart from this difference in expression levels, the two genes are regulated similarly. To study the role of cis-acting elements in the promoter region of the AOX2 gene, we constructed expression plasmids in which the human serum albumin (HSA) gene was placed under the control

of various deleted or ***mutated*** AOX2 ***promoter*** derivatives.

By analyzing the expression of HSA in *P. pastoris* transformants, we have identified three cis-acting regulatory elements in the AOX2 promoter. The positive cis-acting element AOX2-UAS, located between positions -337 and

-313 (relative to the transcription initiation codon), is required for response to transcriptional induction by methanol in an orientation-independent manner, and artificial amplification of the AOX2-UAS resulted in an ***increase*** in the transcriptional ***activity*** of the ***promoter***. A sequence homologous to AOX2-UAS was also found in the AOX1 promoter, and in methanol-regulated

promoters in other methylotrophic yeast. Two negative cis-acting elements,

AOX2-URS1 and AOX2-URS2 play a role in repressing transcription from the

AOX2 promoter. The function of AOX2-UAS is completely repressed by this

unique repression system when both the AOX2-URS1 and AOX2-URS2 are

functional.

L18 ANSWER 32 OF 43 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:531613 HCAPLUS

DOCUMENT NUMBER: 121:131613

TITLE: Activation of the .gamma.E-crystallin pseudogene in the human hereditary Coppock-like cataract

AUTHOR(S): Brakenhoff, Ruud H.; Henskens, Hans A. M.; van Rossum,

Maarten W. P. C.; Lubsen, Nicolette H.; Schoenmakers, John G. G.

CORPORATE SOURCE: Dep. Mol. Cell. Biol., Univ. Nijmegen, Nijmegen, 6525

ED, Neth.

SOURCE: Hum. Mol. Genet. (1994), 3(2), 279-83

CODEN: HMGEES; ISSN: 0964-6906

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The locus for the hereditary human Coppock-like cataract (CCL) is closely

linked to a particular combination of polymorphic TaqI sites within the human .gamma.-crystallin gene cluster. Mapping of these sites shows that they define a 15 kb region encompassing the .gamma.D and .psi..gamma.E gene. The .gamma.D and the .psi..gamma.E gene were cloned

from the CCL chromosome and characterized. The .gamma.D gene was functionally equiv. to its allele cloned from a wild-type chromosome. The CCL .psi..gamma.E gene contains a cluster of sequence changes within and

around its TATA box. Together these cause a 10-fold ***increase*** in

the ***activity*** of the .psi..gamma.E ***promoter***, raising the level of expression of this gene to 30% of that of the .gamma.D gene. The predicted protein product of the .psi..gamma.E gene is a 6 kD N-terminal .gamma.-crystallin fragment. Reactivation of the

.psi..gamma.E

gene and concomitant over-expression of the .gamma.-crystallin fragment could be the cause of the Coppock-like cataract.

L18 ANSWER 33 OF 43 MEDLINE

ACCESSION NUMBER: 93252850 MEDLINE

DOCUMENT NUMBER: 93252850 PubMed ID: 7683658

TITLE: Identification of a promoter element which participates in cAMP-stimulated expression of human insulin-like growth factor-binding protein-1.

AUTHOR: Suwanichkul A; DePaolis L A; Lee P D; Powell D R

CORPORATE SOURCE: Department of Pediatrics, Baylor College of Medicine,

Houston, Texas 77030.

CONTRACT NUMBER: RO1 DK-38773 (NIDDK)

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 May 5) 268 (13)

9730-6.

Journal code: HIV; 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199306

ENTRY DATE: Entered STN: 19930618

Last Updated on STN: 19980206

Entered Medline: 19930604

AB In hepatocytes, insulin-like growth factor-binding protein-1 (IGFBP-1)

levels are increased by glucocorticoids and by agents that raise intracellular cAMP levels such as glucagon, theophylline, forskolin, and cAMP analogues. In contrast, insulin lowers IGFBP-1 levels, an effect dominant over the glucocorticoid and cAMP effects. Previous studies showed

that dibutyl cAMP (Bt2cAMP) and theophylline ***increase*** IGFBP-1

promoter ***activity*** in HEP G2 human hepatoma cells and

that insulin abolishes this increase. In studies reported here, HEP G2 cells were used to further evaluate the role of cAMP in stimulating IGFBP-1 expression. Initial studies found that either 0.5 or 5.0 mM Bt2cAMP alone, or the combination of 0.5 mM Bt2cAMP and 2 mM theophylline, increased IGFBP-1 protein levels, mRNA levels, and promoter activity, but

that the addition of theophylline to Bt2cAMP was required to give a approximately 5-fold ***increase*** in ***promoter*** ***activity***. Deletion ***mutations*** of the IGFBP-1 ***promoter*** were used to show that much of the effect of Bt2cAMP

and theophylline was conferred by the region between 269 and 246 base pairs (bp) 5' of the IGFBP-1 mRNA cap site. DNase I protection assays showed that HEP G2 nuclear extract footprinted the region from 273 to 249 bp 5' of the cap site; this region, designated P2, has a central CGTCA motif common to cAMP-responsive elements (CREs). Mutating the CGTCA motif in the

1205-bp IGFBP-1 promoter construct to TAGCA led to a 51% decrease in the

ability of Bt2cAMP and theophylline to stimulate IGFBP-1 promoter activity

above control levels. In addition, cotransfection of the catalytic subunit of cAMP-dependent protein kinase A (PKA) with the native 1205-bp IGFBP-1

promoter construct stimulated IGFBP-1 promoter activity 3.9-fold, but the TAGCA mutation decreased by 73% the ability of PKA to stimulate IGFBP-1

promoter activity above control levels. ***Mutating*** the CGTCA motif to TAGCA also blocked the ability of both crude HEP G2 nuclear

extract and recombinant CRE-binding protein to bind to the P2 element. These data suggest that the P2 element is a CRE that confers at least part of the stimulatory effect of cAMP on the human IGFBP-1 promoter.

L18 ANSWER 34 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS

DUPLICATE 20

ACCESSION NUMBER: 1993:587793 BIOSIS

DOCUMENT NUMBER: PREV199497007163

TITLE: Transcriptional control of human papillomavirus (HPV) oncogene expression: Composition of the HPV type 18

upstream regulatory region.
 AUTHOR(S): Butz, Karin; Hoppe-Seyler, Felix (1)
 CORPORATE SOURCE: (1) Projektgruppe Angewandte Tumorstudiologie,
 Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 242, D-69120
 Heidelberg Germany
 SOURCE: Journal of Virology, (1993) Vol. 67, No. 11, pp.
 6476-6486.
 ISSN: 0022-538X.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 AB The malignant transformation potential of high-risk human
 papillomaviruses
 (HPVs) is closely ***linked*** to the expression of the viral E6 and
 E7 genes. To elucidate the molecular mechanisms resulting in HPV
 oncogene
 expression, a systematic analysis of the cis-regulatory elements within
 the HPV type 18 (HPV18) upstream regulatory region (URR) which
 regulate
 the activity of the E6/E7 promoter was performed. As the functional
 behavior of a given cis-regulatory element can be strongly influenced by
 the overall composition of a transcriptional control region, individual
 elements were inactivated by site-directed mutagenesis in the
 physiological context of the complete HPV18 URR. Subsequently, the
 effects
 of these mutations on the activity of the E6/E7 promoter were assessed by
 transient transfection assays. We found that the transcriptional
 stimulation of the E6/E7 promoter largely depends on the integrity of
 cis-regulatory elements bound by AP1, Sp1, and in certain epithelial
 cells, KRF-1. In contrast to previous reports implying a key role for NF1
 and Oct-1 recognition motifs in the stimulation of papillomavirus
 oncogene
 expression, the inactivation of these elements in the context of the HPV18
 URR did not strongly affect the transcriptional activity of the E6/E7
 promoter. ***Mutation*** of a ***promoter***-proximal
 glucocorticoid response element completely abolished dexamethasone
 inducibility of the HPV18 E6/E7 ***promoter*** and resulted in an
 increase of its basal ***activity***. Functional dissection of
 the HPV18 constitutive enhancer region indicates that its transcriptional
 activity is largely generated by functional synergism between a centrally
 located AP1 module and thus far undetected cis-active elements present in
 the 5' flank of the enhancer. Furthermore, comparative analyses using
 homologous and heterologous promoters show that the transcriptional
 activity of HPV18 enhancer elements is influenced by the nature of the
 test promoter in a cell-type-specific manner.

L18 ANSWER 35 OF 43 EMBASE COPYRIGHT 2002 ELSEVIER SCI.
 B.V.DUPLICATE 21
 ACCESSION NUMBER: 94209393 EMBASE
 DOCUMENT NUMBER: 1994209393
 TITLE: Comparative studies of nondeletional HPFH .gamma.-globin
 gene promoters.
 AUTHOR: Motum P.J.; Lindeman R.; Harvey M.P.; Trent R.J.
 CORPORATE SOURCE: Department Molecular Genetics, Royal Prince
 Alfred Hospital, Camperdown, NSW 2050, Australia
 SOURCE: Experimental Hematology, (1993) 21/7 (852-858).
 ISSN: 0301-472X CODEN: EXHEBH
 COUNTRY: United States
 DOCUMENT TYPE: Journal; Article
 FILE SEGMENT: 022 Human Genetics
 025 Hematology
 LANGUAGE: English
 SUMMARY LANGUAGE: English
 AB The -198 T->C and the -175 T->C transitions involving the proximal
 .gamma.-globin gene promoter are associated with the hereditary
 persistence of fetal hemoglobin (HPFH) phenotype and have been
 demonstrated to ***increase*** ***promoter*** ***activity***
 in erythroid cells using transient and stable transfection systems. The
 above base changes are thought to alter the binding of different
 transcription regulatory proteins. Another ***mutation*** of the
 proximal .gamma.-globin ***promoter***, -158 C->T, has been less
 clearly ***linked*** to the HPFH phenotype but has been associated
 with increased G. gamma. activity. In the present paper, the -198 T->C,
 -175 T->C and -158 C->T mutations both singly and in various
 combinations
 were evaluated by an in vitro expression assay. .gamma.-Globin promoters
 were transfected by electroporation into K562 human erythroleukemia

cells
 and their activity measured in a human growth hormone (hGH) reporter
 gene
 assay. A novel cotransfectant was used to assess transfection efficiency.
 Results confirmed the previously reported upregulation of .gamma.-globin
 activity with the -198 T->C and -175 T->C HPFH ***mutations*** and
 a
 cooperative effect on ***promoter*** activity when both these
 mutations are present in cis. No effect of the -158 C->T mutation
 was seen either alone or in combination with the -175 T->C and -198
 T->C
 mutations.

L18 ANSWER 36 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 22
 ACCESSION NUMBER: 1992:325796 BIOSIS
 DOCUMENT NUMBER: BA94:27637
 TITLE: PROMOTER ELEMENTS REQUIRED FOR POSITIVE
 CONTROL OF
 TRANSCRIPTION OF THE ESCHERICHIA-COLI UHP
 GENE.
 AUTHOR(S): MERKEL T J; NELSON D M; BRAUER C L;
 KADNER R J
 CORPORATE SOURCE: MOL. BIOL. INST., UNIV. VA.,
 CHARLOTTESVILLE, VA. 22908.
 SOURCE: J BACTERIOL, (1992) 174 (9), 2763-2770.
 CODEN: JOBAAY. ISSN: 0021-9193.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB The uhpABC locus of Escherichia coli encodes the transport system
 which
 allows the cell to accumulate a variety of sugar phosphates in unaltered
 form. The expression of uhpT, the gene encoding the transport protein, is
 regulated by the uhpABC gene products. The UhpA protein is required for
 expression; its deduced amino acid sequence shows that it belongs to a
 subfamily of bacterial transcription regulators including NarL, DegU, and
 FixJ. Members of this subfamily have an amino-terminal phosphorylation
 domain characteristic of so-called two-component regulators, such as
 OmpR,
 CheY, PhoB, and NtrC, and a carboxyl-terminal domain conserved among
 many
 transcriptional activators, including LuxR and MalT. The major sequence
 elements in the uhpT promoter that are needed for uhpT expression were
 investigated. Northern (RNA) hybridization analysis showed that the uhpT
 transcript was only present in cells induced for UhpT transport activity.
 The start site of transcription was identified by primer extension.
 Comparison of the regions upstream of the uhpT transcription start site in
 E. coli and Salmonella typhimurium suggested the presence of four
 sequence
 elements that might be involved in promoter function: a typical -10
 region, a short inverted repeat centered at -32, a long inverted repeat
 centered at -64, and a cyclic AMP receptor protein-binding sequence
 centered at -103. Deletion and ***linker*** substitution
 mutations in the ***promoter*** demonstrated that the
 presence
 of the cyclic AMP receptor protein-binding site resulted in about an
 eightfold ***increase*** in ***promoter*** ***activity*** and
 that the -64, -32, and -10 elements were essential for promoter function.
 In vivo titration of transcriptional activator UhpA by the intact or
 mutant promoters on multicopy plasmids identified the -64 element as the
 UhpA-binding site. The two halves of the -64 inverted repeat did not
 contribute equally to promoter function and did not have to be intact for
 UhpA titration. The sequence recognized by UhpA is predicted to be
 5'-GGCAAAACNNNGAAA.

L18 ANSWER 37 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 23
 ACCESSION NUMBER: 1991:369135 BIOSIS
 DOCUMENT NUMBER: BA92:57360
 TITLE: EVIDENCES AGAINST A ROLE FOR ADENINE
 METHYLATION IN THE
 TRYPTOPHAN BIOSYNTHETIC PATHWAY IN
 ESCHERICHIA-COLI AND FOR
 A GROWTH PHASE-DEPENDENT INDUCTION OF THE
 TRP PROMOTER.
 AUTHOR(S): BARRAS F; MAGNAN M; MARINUS M G
 CORPORATE SOURCE: LAB. CHIM. BACTERIENNE, CNRS, 31
 CHEMIN JOSEPH AIGUIER,

13274 MARSEILLE CEDEX 9, FR.
 SOURCE: CURR MICROBIOL, (1991) 23 (1), 21-26.
 CODEN: CUMIDD. ISSN: 0343-8651.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB Dam-mediated adenine methylation at GATC sites can interfere with gene expression. By use of lacZ ***fusion*** technology, the efficiency of trpR and trpS promoters (which contain a GATC site) and of trp (the target of TrpR repressor) was analyzed in dam+ and dam- back-grounds. In exponentially growing cells, the dam mutation leads to an ***increased*** ***activity*** of trpR ***promoter*** but does not affect trpS or trp promoters. The DAM-mediated induction of trpR was, however, found to be repressed by trpR-mediated autoregulation. In contrast, trp-lacZ directed .beta.-galactosidase activity was increased at least sixfold in dam- cells in late logarithmic growth phase. In dam+ cells, expression of trp-lacZ was similarly late-growth-phase induced, albeit to a reduced extent. Hence, we propose that enhancement of growth phase-dependent gene induction constitutes a previously unidentified trait of dam mutation. This finding is discussed in the context of the pleiotropic phenotype of dam mutation.

L18 ANSWER 38 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 24
 ACCESSION NUMBER: 1991:3888 BIOSIS
 DOCUMENT NUMBER: BA91:3888
 TITLE: ESCHERICHIA-COLI FIS PROTEIN ACTIVATES RIBOSOMAL RNA TRANSCRIPTION IN-VITRO AND IN-VIVO.
 AUTHOR(S): ROSSE W; THOMPSON J F; NEWLANDS J T; GOURSE R L
 CORPORATE SOURCE: DEP. BACTERIOL., UNIV. WIS., 1550 LINDEN DR., MADISON, WIS. 53706.
 SOURCE: EMBO (EUR MOL BIOL ORGAN) J, (1990) 9 (11), 3733-3742.
 CODEN: EMJODG. ISSN: 0261-4189.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB An upstream activation region (UAR) contributes to the extremely high activity of the Escherichia coli ribosomal RNA ***promoter***, rrmB P1, ***increasing*** its ***activity*** 20- to 30-fold over that of the same promoter lacking the UAR. We have used DNase footprinting to define three specific sites in the rrmB P1 UAR that bind Fis, a protein identified previously by its role in recombinational enhancer function in other systems. We find that purified Fis activates transcription from promoters containing these sites 10- to 20-fold in vitro at concentrations correlating with the filling of these sites. Three approaches indicate that Fis contributes to the function of the UAR in vivo. First, there is a progressive loss in the activity of rrmB P1-lacZ ***fusions*** as Fis binding sites are deleted. Second, an rrmB P1 ***promoter*** with a ***mutation*** in a Fis binding site has 5-fold reduced transcription activity in vivo, dramatically reduced Fis binding in vitro, and shows no Fis dependent transcription activation in vitro. Third, upstream activation is reduced 5-fold in a fis- strain. We show that rRNA promoters derepress in response to the loss of Fis in vivo in accord with the predictions of the negative feedback model for rRNA regulation. We find that fis is not essential for the function of two control systems known to regulate rRNA, growth rate dependent control and stringent control. On the basis of these results, we propose roles for Fis and the upstream activation system in rRNA synthesis.

L18 ANSWER 39 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 25
 ACCESSION NUMBER: 1990:69504 BIOSIS
 DOCUMENT NUMBER: BA89:37330
 TITLE: CELL-TYPE SPECIFICITY OF REGULATORY ELEMENTS IDENTIFIED BY ***LINKER*** SCANNING MUTAGENESIS IN THE PROMOTER OF THE CHICKEN LYSOZYME GENE.
 AUTHOR(S): LUCKOW B; SCHUETZ G
 CORPORATE SOURCE: INST. FUER ZELL- UND TUMORBIOL., DEUTSCHES

KREBSFORSCHUNGSZENTRUM, IM NEUENHEIMER FELD 280, D-6900 HEIDELBERG 1, W. GER.
 SOURCE: NUCLEIC ACIDS RES, (1989) 17 (21), 8451-8462.
 CODEN: NARHAD. ISSN: 0305-1048.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB The chicken lysozyme gene is constitutively expressed in macrophages, in oviduct cells its expression is controlled by steroid hormones, and in fibroblasts the gene is not expressed. A ***fusion*** gene consisting of promoter sequences of the lysozyme gene from -208 to +15 in front of the chloramphenicol acetyltransferase (CAT) coding region was more than 50 times less active in non-expressing cells as compared to expressing cells. In order to identify the element(s) responsible for this cell-type specificity 31 different ***linker*** scanning ***mutations*** were generated within this ***promoter*** fragment and analyzed by transient transfections in the three types of chicken cells mentioned above. Three mutation sensitive regions located around position -25, -100 and between -158 and -208 were detected in each cell type, however, several LS mutations displayed clear cell-type specific differences in their phenotypic effects. Interestingly, a few LS ***mutations*** led to an ***increase*** in ***promoter*** ***activity*** in fibroblasts suggesting that the corresponding wildtype sequences represent binding sites for negatively acting transcription factors.

L18 ANSWER 40 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 26
 ACCESSION NUMBER: 1989:471731 BIOSIS
 DOCUMENT NUMBER: BA88:107491
 TITLE: IDENTIFICATION OF PROMOTER MUTANTS DEFECTIVE IN GROWTH-RATE-DEPENDENT REGULATION OF RIBOSOMAL RNA TRANSCRIPTION IN ESCHERICHIA-COLI.
 AUTHOR(S): DICKSON R R; GAAL T; DEBOER H A; DEHASETH P L; GOURSE R L
 CORPORATE SOURCE: DEP. BACTERIOL., UNIV. WISCONSIN, 1550 LINDEN DRIVE, MADISON, WIS. 53706.
 SOURCE: J BACTERIOL, (1989) 171 (9), 4862-4870.
 CODEN: JOBAAY. ISSN: 0021-9193.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB We measured the activities of 50 operon ***fusions*** from a collection of mutant and wild-type rrmB P1 (rrmB1p in the nomenclature of B. J. Bachmann and K. B. Low [Microbiol. Rev. 44:1-56, 1980]) promoters under different nutritional conditions in order to analyze the DNA sequence determinants of growth rate-dependent regulation of rRNA transcription in Escherichia coli. Mutants which deviated from the wild-type -10 or -35 hexamers or from the wild-type 16-base-pair spacer length between the hexamers were unregulated, regardless of whether the ***mutations*** brought the ***promoters*** closer to the E. coli ***promoter*** consensus sequence and ***increased*** ***activity*** or whether the changes took the promoters further away from the consensus and reduced activity. These data suggest that rRNA promoters have evolved to maintain their regulatory abilities rather than to maximize promoter strength. Some double substitutions outside the consensus hexamers were almost completely unregulated, while single substitutions at several positions outside the -10 and -35 consensus hexamers exerted smaller but significant effects on regulation. These studies suggest roles for specific promoter sequences and/or structures in interactions with regulatory molecules and suggest experimental tests for models of rRNA regulation.

L18 ANSWER 41 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 ACCESSION NUMBER: 1989:181517 BIOSIS
 DOCUMENT NUMBER: BA87:92783
 TITLE: THE HUMAN THYMIDINE KINASE GENE PROMOTER DELETION ANALYSIS AND SPECIFIC PROTEIN BINDING.
 AUTHOR(S): ARCOT S S; FLEMINGTON E K; DEININGER P L
 CORPORATE SOURCE: DEP. OF BIOCHEM. MOL. BIOL., LA. STATE UNIV. MED. CENT., 1901 PERDIDO ST., NEW ORLEANS, LA. 70112.
 SOURCE: J BIOL CHEM, (1989) 264 (4), 2343-2349.

CODEN: JBCHA3. ISSN: 0021-9258.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB We report a functional analysis of the human thymidine kinase (tk) gene promoter. We have ***linked*** the tk promoter to the chloroamphenicol acetyltransferase (CAT) gene to allow direct measurement of promoter strength by assaying chloroamphenicol acetyltransferase enzyme activity after transfection into mouse L cells. Putative transcription elements have been identified by deletion and ***mutation*** analysis of this ***promoter***. The ***promoter*** relies primarily on two "CCAAT" elements and a series of "GC" elements found farther upstream.
 Two-thirds of promoter activity is maintained by a construct containing 139 base pairs of sequence upstream of the initiation of transcription that contains only one GC and one of the CCAAT elements. In addition, an evolutionary comparison identifies two highly conserved promoter elements:
 the -40 CCAAT element and a "TATA" element located at -21. We have further characterized both CCAAT elements using a mutational as well as protein binding analysis. From this study we have determined that both the -70 and -40 CCAAT elements bind strongly to the same factor, with a slightly higher affinity for the -40 CCAAT. Competition studies suggest that the CCAAT factor that binds to this promoter is homologous to protein nuclear factor Y, which binds to the major histocompatibility complex class III E.alpha. gene promoter. In addition, either CCAAT element is capable for supplying almost as much promoter strength as is supplied in the presence of both.

L18 ANSWER 42 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 27
 ACCESSION NUMBER: 1988:417176 BIOSIS
 DOCUMENT NUMBER: BA86:79788
 TITLE: IDENTIFICATION OF A DOWNSTREAM SEQUENCE AND BINDING PROTEIN THAT REGULATE ADENOVIRUS MAJOR LATE PROMOTER TRANSCRIPTION IN-VITRO.
 AUTHOR(S): COHEN R B; YANG L; THOMPSON J A; SAFER B
 CORPORATE SOURCE: SECTION RNA AND PROTEIN BIOSYNTHESIS, LAB. MOLECULAR HEMATOL., NATL. HEART LUNG AND BLOOD INST., NATL. INSTITUTES HEALTH, BETHESDA, MARYLAND 20892.
 SOURCE: J BIOL CHEM, (1988) 263 (21), 10377-10385.
 CODEN: JBCHA3. ISSN: 0021-9258.

FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB Gel electrophoresis mobility shift and DNase I footprint assays detect a cellular nuclear protein in extracts made from uninfected human cells which binds to a downstream promoter sequence (DPS) in the human adenovirus 2 major late ***promoter***. By DNase I footprint and ***mutation*** analyses, we have determined that this new regulatory element extends from positions + 146 + 165 (relative to the cap site at position +1). We show by UV cross- ***linking*** that a 40-kDa polypeptide specifically binds to this region. Mutations within the DPS which decrease protein binding by 80-90% also cause a 2.5-3-fold decrease in in vitro major late promoter transcription efficiency. Alteration of the template in the 5'-flanking region of the DPS does not affect nuclear protein binding or transcription efficiency. Interestingly, a T.fwdarw. G transversion at position + 160 which ***increases*** protein binding also impairs ***promoter*** ***activity***.

L18 ANSWER 43 OF 43 BIOSIS COPYRIGHT 2002 BIOSIS
 DUPLICATE 28
 ACCESSION NUMBER: 1988:374436 BIOSIS
 DOCUMENT NUMBER: BA86:58346
 TITLE: A NEW ***HYBRID*** ***PROMOTER*** AND ITS EXPRESSION VECTOR IN ESCHERICHIA-COLI.
 AUTHOR(S): SHIBUI T; UCHIDA M; TERANISHI Y
 CORPORATE SOURCE: BIOSCIENCES LAB., RES. CENT., MITSUBISHI CHEMICAL

INDUSTRIES, 1000 KAMOSHIDA-CHO, MIDORI-KU, YOKOHAMA-SHI, KANAGAWA 227, JPN.
 SOURCE: AGRIC BIOL CHEM, (1988) 52 (4), 983-988.
 CODEN: ABCHA6. ISSN: 0002-1369.
 FILE SEGMENT: BA; OLD
 LANGUAGE: English
 AB A new ***hybrid*** ***promoter***, "pac", comprising the '-35' region of the bacteriophage T5 P25 gene promoter and the '-10' and the operator regions of the lacUV5 promoter, was chemically synthesized and used to construct a new expression vector. The ***activity*** of the ***hybrid*** ***promoter*** was ***compared*** with that of the tac (trp: lac ***fusion***) promoter, which is widely used as a strong and controllable promoter. The activity of the pac promoter was found to be stronger by about 3-fold than that of tac when assayed with the chloramphenicol acetyltransferase (CAT) system. The pac promoter, however, was not repressed as efficiently as the tac promoter.

09/28/255
A#6

1. Document ID: US 20010049118 A1

L9: Entry 1 of 23

File: PGPB

Dec 6, 2001

PGPUB-DOCUMENT-NUMBER: 20010049118
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010049118 A1

TITLE: Multiply transformed koji mold and a method of manufacturing a flavor enhancer using the same

PUBLICATION-DATE: December 6, 2001
US-CL-CURRENT: 435/41; 426/46, 435/254.11

APPL-NO: 09/ 801734
DATE FILED: March 9, 2001

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

APPL-NO	DOC-ID	APPL-DATE
JP	64739/2000	
	2000JP-64739/2000	March 9, 2000

IN: Umitsuki, Genryou, Sato, Hiroe, Sugishita, Misao, Fukushima, Yaichi, Koyama, Yasuji

AB: The present invention provides a koji mold having increased protease activity and peptidase activity relative to a parent strain, a method of breeding the koji mold, and a method of manufacturing a flavor enhancer using the koji mold. More specifically, the present invention provides: (1) a koji mold having increased protease activity and peptidase activity relative to a parent strain obtained by transformation using a protease gene and a peptidase gene, (2) a method of breeding the above koji mold which comprises transforming a parent strain of koji mold using a protease gene and a peptidase gene, and then selecting a transformant having higher protease activity and peptidase activity relative to a parent strain, (3) a method of manufacturing a flavor enhancer which comprises allowing a culture product of the above koji mold to act on a protein.

L9: Entry 1 of 23

File: PGPB

Dec 6, 2001

DOCUMENT-IDENTIFIER: US 20010049118 A1
TITLE: Multiply transformed koji mold and a method of manufacturing a flavor enhancer using the same

BSTX:
[0025] The term "protease gene" and "peptidase gene" include nucleic acid sequences which when introduced into a parent strain by transformation, can increase the activity of each enzyme of the koji mold. Such nucleic acid sequences include a structural gene of an enzyme, a mutant gene in which a mutation has been introduced within a structural gene to increase the activity of the enzyme itself, expression regulation sites (promoter, terminator, enhancer, etc.) of the structural gene, mutant expression regulation sites, expression regulation sites of different

genes, or derivatives thereof, and a nucleic acid sequence consisting of a plurality of these sequences linked together.

Further, the nucleic acid sequence can be of natural derivation or a synthetic product, and for example, genomic DNA, cDNA, PCR fragment, chemical synthetic/semi-synthetic DNA and the like can be used.

2. Document ID: US 6322962 B1 ✓

L9: Entry 2 of 23

File: USPT

Nov 27, 2001

US-PAT-NO: 6322962
DOCUMENT-IDENTIFIER: US 6322962 B1
TITLE: Sterol-regulated Site-1 protease and assays of modulators thereof
DATE-ISSUED: November 27, 2001

US-CL-CURRENT: 435/4; 435/320.1, 435/325, 435/455, 435/6, 530/350, 536/23.1, 536/23.4

APPL-NO: 9/ 360237
DATE FILED: July 23, 1999

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application, Ser. No. 60/096,571, filed Aug. 14, 1998. The government owns rights in the present invention pursuant to grants number HL-20948 from the National Institutes of Health, NIH Research Science Fellowship Award number HL09993, and NIH Medical Scientist Training Grant GM08014.

IN: Brown; Michael S., Cheng; Dong, Espenshade; Peter J., Goldstein; Joseph L., Rawson; Robert B., Sakai; Juro

AB: The invention provides assays for the identification of modulators of Site-1 protease. Further provided by the invention are expression constructs and the transgenic cells useful for the development of such assays for Site-1 specific protease. The cells allow the implementation of in vitro assays for potential modulators of Site-specific proteases. Still further provided by the invention are in vitro assays employing Site-1 protease which has been isolated from cells.

L9: Entry 2 of 23

File: USPT

Nov 27, 2001

DOCUMENT-IDENTIFIER: US 6322962 B1
TITLE: Sterol-regulated Site-1 protease and assays of modulators thereof

DRPR:
Modified promoters could also be used with the current invention. Promoters can be modified in a number of ways to increase their transcriptional activity. Multiple copies of a given promoter can be linked in tandem, mutations which increase activity may be introduced, single or multiple copies of individual promoter elements may be attached, parts of unrelated promoters may be fused together,

or some combination of all of the above can be employed to generate highly active promoters. All such methods are contemplated for use in connection with the present invention.

DRPR:

German et al., (1992) mutated three nucleotides in the transcriptionally important FLAT E box of the rat insulin I gene promoter (RIP), resulting in a three- to four-fold increase in transcriptional activity of the mutated RIP compared to that of a nonmutated RIP as assayed in transiently transfected HIT cells. Also, the introduction of multiple copies of a promoter element from the E. coli tetracycline resistance operon promoter were introduced into the CMV promoter, significantly increasing the activity of this already very potent promoter (Liang et al., 1996). Additionally, part of the CMV promoter, which has high but short-lived transcriptional activity in dog myoblasts, was linked to the muscle-specific creatine kinase promoter (MCKp), which has weak but sustained expression in dog myoblasts, resulting in a hybrid promoter that sustained high-level expression for extended periods in dog myoblasts.

3. Document ID: US 6245967 B1

L9: Entry 3 of 23

File: USPT

Jun 12, 2001

US-PAT-NO: 6245967

DOCUMENT-IDENTIFIER: US 6245967 B1

TITLE: Process and DNA molecules for increasing the photosynthesis rate in plants

DATE-ISSUED: June 12, 2001

US-CL-CURRENT: 800/278; 435/320.1, 435/419, 435/468, 435/69.1, 536/23.2, 536/23.5, 536/23.6, 536/23.7, 800/284, 800/288, 800/289, 800/290

APPL-NO: 8/860820

DATE FILED: September 4, 1997

PARENT-CASE:

This application is filed under 35 U.S.C. .sectn.371 as a national stage application of the International application PCT/EP96/00111, filed Jan. 11, 1996, and is entitled to priority under 35 U.S.C. .sectn.119 to German patent application 195 02 053.7, filed Jan. 13, 1995.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY

APPL-NO

APPL-DATE

DE

195 02 053

January 13, 1995

PCT-DATA:

APPL-NO

DATE-FILED

PUB-NO

PUB-DATE

371-DATE

102(E)-DATE

PCT/EP96/00111

January 11, 1996

WO96/21737

Jul 18, 1996

Sep 4, 1997

Sep 4, 1997

IN: Sonnewald; Uwe, Kossmann; Jens, Bowien; Botho

AB: The invention describes recombinant DNA molecules that allow expression of a deregulated or unregulated fructose-1,6-bisphosphatase (FBPase) in plant cells. Such expression leads to an increase in the photosynthesis rate and biomass production in photosynthetically active cells. Furthermore, the invention describes transgenic plants that show an increased photosynthesis rate due to the expression of a deregulated or unregulated FBPase.

L9: Entry 3 of 23

File: USPT

Jun 12, 2001

DOCUMENT-IDENTIFIER: US 6245967 B1

TITLE: Process and DNA molecules for increasing the photosynthesis rate in plants

BSPR:

The mutagenized DNA sequences coding for the FBPase enzymes are subsequently introduced for analysis of the FBPase activity into a suitable host, preferably into an FBPase-deficient E. coli strain. An example of such a strain is E. coli strain DF657 (Sedivy et al., J. Bacteriol. 158 (1984), 1048-1053). For an identification of clones expressing a functional FBPase enzyme the transformed cells are plated onto minimal medium containing, e.g., glycerol and succinate (each in a concentration of 0.4%) as carbon source. Cells that do not express functional FBPase cannot grow on such a medium. A first pointer to the activity of the expressed FBPase can be the growth rates of transformed viable clones. In order to preclude mutations in the promoter region resulting in an increased FBPase activity, the mutated coding DNA sequences that allow growth on a minimal medium have to be recloned into non-mutagenated vectors and again be screened for FBPase activity (again by complementation of a FBPase deficient E. coli strain). Mutants that effect a complementation of an FBPase deficient E. coli strain even in the second screening round are used for the analysis of FBPase activity in the presence of various inhibitors and activators.

4. Document ID: US 6214614 B1

L9: Entry 4 of 23

File: USPT

Apr 10, 2001

US-PAT-NO: 6214614

DOCUMENT-IDENTIFIER: US 6214614 B1

TITLE: Cell cycle regulated repressor and DNA element

DATE-ISSUED: April 10, 2001

US-CL-CURRENT: 435/320.1; 536/24.1

APPL-NO: 8/ 793660
DATE FILED: September 9, 1997

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

	APPL-NO	APPL-DATE
GB	9417366	August 26, 1994
GB	9506466	March 29, 1995

PCT-DATA:
APPL-NO

	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/GB95/02000	August 23, 1995	WO96/06943	Mar 7, 1996	Sep 9, 1997	Sep 9, 1997

IN: Muller; Rolf

AB: The present invention relates to a cell cycle regulated repressor protein which binds to a DNA element present in the control sequences of the human cdc25C gene and other cell cycle regulated genes, as well as the use thereof in cell cycle regulated expression systems.

L9: Entry 4 of 23

File: USPT

Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214614 B1
TITLE: Cell cycle regulated repressor and DNA element

DRPR:

Transient expression analysis of a cdc25C promoter-luciferase construct containing a mutated CDE (construct C74R1) in quiescent (G.sub.0) versus stimulated (G.sub.2) NIH3T3 cells (FIG. 7A, left graph) and in quiescent (G.sub.0) versus growing cells (panel A, right graph). The CDE sequence was mutated as follows: . . . CTG GCGGAA . . . fwdarw. . . CTGATCAA . . . (protected G residues underlined; mutated bases double-underlined). FIG. 7B shows the results obtained in 6 independent experiments. Values separated by slashes indicate luciferase activities obtained with C74 (left value) and V74R1 (right value), respectively, under different growth conditions (top panel: G.sub.0 and growing; bottom panel: G.sub.0 and G.sub.2). The increase in promoter activity caused by the CDE mutation is also indicated for each pair of values (fold increase). Averages and standard deviations for G.sub.0 and G.sub.2 /growing cells are shown at the bottom, indicating that mutation of the CDE in C74R1 led to an average 12.8-fold increase in G.sub.0 cells, but only to 1.6-fold increase in G.sub.2 /growing cells.

5. Document ID: US 6207455 B1

L9: Entry 5 of 23

File: USPT

Mar 27, 2001

US-PAT-NO: 6207455
DOCUMENT-IDENTIFIER: US 6207455 B1
TITLE: Lentiviral vectors
DATE-ISSUED: March 27, 2001

US-CL-CURRENT: 435/457; 435/320.1, 435/325, 435/363, 435/366, 435/368, 435/369, 435/370, 435/371, 435/372, 435/455, 435/456

APPL-NO: 8/ 935312
DATE FILED: September 22, 1997

PARENT-CASE:

This application is a continuation-in-part of Chang, Ser. No. 08/848,760, filed May 1, 1997, now pending.

IN: Chang; Lung-Ji

AB: The present invention contemplates novel lentiviral vectors which exhibit strong promoter activity in human and other cells. Vectors are provided which are packaged efficiently in packaging cells and cell lines to generate high titer recombinant virus stocks. The present invention further relates to HIV vaccines and compositions for gene therapy. In particular, the present invention provides attenuated replication-competent HIV vaccines and replication-defective HIV vectors.

L9: Entry 5 of 23

File: USPT

Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207455 B1
TITLE: Lentiviral vectors

DEPR:

It was also found that several LTR deletion mutants containing a cytomegalovirus enhancer element were capable of attenuating HIV-1 (i.e., the mutants were capable of infecting human lymphocytes with reduced cytopathic effects when the gene also was deleted). Instead of killing the entire culture, infection with these LTR and tat mutants led to rapid cell recovery and establishment of persistent infection. The replication efficiency was not markedly affected by these mutations. By mutating the tat gene, it was also found that the recombinant LTRs (CMV-IE-HIV-LTR) exhibited increased basal levels of promoter activity which could support virus replication without Tat (L. -J. Chang, and C. Zhang, Virol., 211:157-169[1995]; and D. Robinson et al., Gene Ther., 2:269-278 [1995]). These different HIV-1 mutant constructs were useful for the development of lentiviral vectors.

6. Document ID: US 6177242 B1

L9: Entry 6 of 23

File: USPT

Jan 23, 2001

US-PAT-NO: 6177242

DOCUMENT-IDENTIFIER: US 6177242 B1

TITLE: Genomic DNA fragments containing regulatory and coding sequences for the .beta.2-subunit of the neuronal nicotinic acetylcholine receptor and transgenic animals made using these fragments or mutated fragments
DATE-ISSUED: January 23, 2001

US-CL-CURRENT: 435/6; 435/235.1, 435/320.1, 435/325, 530/350, 536/23.1, 536/23.5, 536/24.1

APPL-NO: 8/ 358627

DATE FILED: December 14, 1994

IN: Changeux; Jean-Pierre, Picciotto; Marina, Bessis; Alain

AB: Several genes encoding subunits of the neuronal nicotinic acetylcholine receptors have been cloned and regulatory elements involved in the transcription of the .alpha.:2 and .alpha.:7-subunit genes have been described. Yet, the detailed mechanisms governing the neuron-specific transcription and the spatio-temporal expression pattern of these genes remain largely uninvestigated. The .beta.2-subunit is the most widely expressed neuronal nicotinic receptors subunit in the nervous system. We have studied the structural and regulatory properties of the 5' sequence of this gene. A fragment of 1163 bp of upstream sequence is sufficient to drive the cell-specific transcription of a reporter gene in both transient transfection assays and in transgenic mice. Deletion analysis and site-directed mutagenesis of this promoter reveal two negative and one positive element. The positively acting sequence includes one functional E-box. One of the repressor elements is located in the transcribed region and is the NRSE/RE1 sequence already described in promoters of neuronal genes.

L9: Entry 6 of 23

File: USPT

Jan 23, 2001

DOCUMENT-IDENTIFIER: US 6177242 B1

TITLE: Genomic DNA fragments containing regulatory and coding sequences for the .beta.2-subunit of the neuronal nicotinic acetylcholine receptor and transgenic animals made using these fragments or mutated fragments

BSPR:

An NRSE/RE1 element is located at the 3' extremity of the promoter. This element has already been shown to restrict the activity of promoters in neuronal cells (Kraner et al., 1992; Mori et al., 1992; Li et al., 1993). In the 1163 bp promoter of the .beta.2-subunit gene, point mutation of this sequence leads to a .about.100 fold increase of the transcriptional activity in fibroblasts implying that this sequence is involved in the neuron-specific expression of the .beta.2-subunit gene. Moreover, sequence comparison shows that this sequence is highly conserved in rat and human .beta.2-subunit cDNAs (Deneris et al., 1988; Anand and Lindstrom, 1990)

as well as in several promoters of genes expressed in the nervous system, such as the middle-weight neurofilament gene, the CAM-L1 gene, the Calbinbin gene, or the cerebellar Ca-binding protein gene (see Table 1B).

DEPR:

An NRSE/RE1 sequence is also present in the proximal region and has been shown to act as a silencer in fibroblasts but not in PC12 cells or neuroblastomas (Kraner et al., 1992; Li et al., 1993; Mori et al., 1992). Point mutation of this sequence in the context of the 1163 bp promoter resulted in a 105-fold increase in the transcriptional activity in fibroblasts, and only a 3-fold increase in neuroblastomas (Table 1A). This sequence is thus responsible for at least part of the cell-specific expression of the .beta.2 subunit gene.

7. Document ID: US 6162641 A

L9: Entry 7 of 23

File: USPT

Dec 19, 2000

US-PAT-NO: 6162641

DOCUMENT-IDENTIFIER: US 6162641 A

TITLE: Neuregulin response element and uses therefor
DATE-ISSUED: December 19, 2000

US-CL-CURRENT: 435/325; 435/320.1, 435/348, 435/349, 435/352, 435/363, 435/368, 435/371, 536/23.1, 536/24.1

APPL-NO: 9/ 092636

DATE FILED: June 5, 1998

PARENT-CASE:

RELATED APPLICATION This application claims the benefit of priority under 35 U.S.C. 19(e) to co-pending U.S. Provisional application Ser. No. 60/048,847, filed Jun. 6, 1997, entitled "Neuregulin Response Element and Uses Therefor" the entire contents of which are hereby incorporated by reference.

IN: Goldman; Daniel, Sapru; Mohan K.

AB: Methods for therapeutics and for screens are provided using a 15 bp sequence in the rat .epsilon.-subunit promoter that regulates PTPase, neuregulin and Ras-dependent gene expression. As this 15 bp sequence is necessary also for low .epsilon.-subunit gene expression in extrajunctional regions of the muscle fiber, the screens can identify agents that simultaneously and oppositely modulate expression in .epsilon.-subunit expression of synaptic and extrajunctional regions.

L9: Entry 7 of 23

File: USPT

Dec 19, 2000

DOCUMENT-IDENTIFIER: US 6162641 A

TITLE: Neuregulin response element and uses therefor

DEPR:

Mutant .epsilon.-108 had less promoter activity than the full length construct (Walke, Wet al. (1994) J. Biol. Chem. 269, 19447-19456). However, promoter mutations .epsilon.DELTA.-56/-67, .epsilon.MUT1, .epsilon.MUT2 and the 3' deletion mutations exhibited an increase in promoter activity (values presented below), indicating that an effect of these deletions is the removal of at least one or several negative elements.

8. Document ID: US 6156509 A

L9: Entry 8 of 23

File: USPT

Dec 5, 2000

US-PAT-NO: 6156509

DOCUMENT-IDENTIFIER: US 6156509 A

TITLE: Method of increasing efficiency of directed evolution of a gene using phagemid

DATE-ISSUED: December 5, 2000

US-CL-CURRENT: 435/6; 435/183, 435/235.1, 435/441, 435/446, 530/350

APPL-NO: 8/ 968627

DATE FILED: November 12, 1997

IN: Schellenberger; Volker

AB: An improved method of mutating DNA is provided comprising mutating a gene bearing phagemid in a host and infecting the host cell with a suitable helper phage to initiate packaging of the DNA into a phagemid. The packaged phagemid is then used to infect a second host cell. The method facilitates more efficient screening for genetic diversity. Also, the gene of interest is mutated and separable from the host cell which undergoes the mutational event reducing false positive results or other artifacts of general mutation techniques.

L9: Entry 8 of 23

File: USPT

Dec 5, 2000

DOCUMENT-IDENTIFIER: US 6156509 A

TITLE: Method of increasing efficiency of directed evolution of a gene using phagemid

DEPR:

Two clones were isolated from population G305 and the TEM-1 gene was sequenced. Both clones carried the same 4 mutations. One of the mutations was silent. The other three lead to amino acid changes

E104K, G238S, and T265M. All three mutations have been previously found in the clinical isolate

TEM-4 (Philippon et al., Antimicrob. Agents Chemother., 1989, Vol. 33, pp.1131-1136). Mutations E1

04K and G238S have also been identified previously by gene shuffling (Stemmer, Nature, 1994, Vol.

370, pp.389-391). The isolate clones had no mutations in the promoter which indicates that the

improvements in the MICs are mainly due to an increase in the specific activity of .beta.-lactamase

for ctx and not due to increased levels of expression.

9. Document ID: US 6083690 A

L9: Entry 9 of 23

File: USPT

Jul 4, 2000

US-PAT-NO: 6083690

DOCUMENT-IDENTIFIER: US 6083690 A

TITLE: Methods and compositions for identifying osteogenic agents

DATE-ISSUED: July 4, 2000

US-CL-CURRENT: 435/6; 435/29, 435/320.1, 435/325, 435/375, 435/4, 435/455, 536/23.1, 536/24.1

APPL-NO: 8/ 458434

DATE FILED: June 2, 1995

IN: Harris; Stephen E., Mundy; Gregory R., Ghosh-Choudhury; Nandini, Feng; Jian Q.

AB: Methods and compositions for identifying osteogenic agents are disclosed, wherein a bone morphogenetic protein promoter is utilized in an assay system to modulate the production of an assayable product of a reporter gene.

L9: Entry 9 of 23

File: USPT

Jul 4, 2000

DOCUMENT-IDENTIFIER: US 6083690 A

TITLE: Methods and compositions for identifying osteogenic agents

DEPR:

The promoters of the genes for BMP-4 and BMP-2 are complex promoters which can be linked to reporter genes, such as e.g. the firefly luciferase gene. When these hybrid genes (for example, bone cell

BMP-4 promoter or bone cell BMP-2 promoter and firefly luciferase, chloramphenicol acetyl

transferase (CAT) cDNAs, or cDNA's for other reporter genes such as .beta.-galactosidase, green

fluorescent protein, human growth hormone, alkaline phosphatase, .beta.-glucuronidase, and the like)

are transfected into bone cells, osteogenic agents which activate the BMP-4 or BMP-2 promoters can

be identified by their capacity in vitro to increase luciferase activity in cell lysates after cell

culture with the agent.

10. Document ID: US 6063598 A ✓

L9: Entry 10 of 23

File: USPT

May 16, 2000

US-PAT-NO: 6063598

DOCUMENT-IDENTIFIER: US 6063598 A

TITLE: Strong homologous promoter obtained from hamsters
DATE-ISSUED: May 16, 2000

US-CL-CURRENT: 435/69.1; 435/320.1, 435/325, 435/455, 536/23.1, 536/24.1

APPL-NO: 9/ 051969
DATE FILED: September 30, 1998

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

APPL-NO	APPL-DATE
DE	
195 39 493	October 24, 1995

PCT-DATA:
APPL-NO

DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/EP96/04631				
October 24, 1996				
	WO97/15664			
		May 1, 1997		
		Sep 30, 1998		
			Sep 30, 1998	

IN: Enenkel; Barbara, Gannon; Frank, Bergemann; Klaus, Noe; Wolfgang

AB: The invention relates to a strong homologous promoter from hamsters. In particular, it relates to the promoter of a gene which codes for the Ubiquitin-S27a-fusion protein. The promoter can be used in processes for preparing heterologous gene products in culture cells, particularly CHO cells.

L9: Entry 10 of 23

File: USPT
May 16, 2000

DOCUMENT-IDENTIFIER: US 6063598 A

TITLE: Strong homologous promoter obtained from hamsters

BSPR:

The promoter sequences described can be functionally linked with other regulatory sequences in an expression cassette. For example, they may be functionally linked to enhancer sequences and in this way the transcription activity is increased. There may be one or more enhancers and/or a number of copies of an enhancer sequence. It is possible to use a CMV- or an SV40-enhancer, for example. Human CMV-enhancer is among the most powerful enhancers identified hitherto. An example of an inducible enhancer is the metallothionein enhancer which can be stimulated by glucocorticoids or heavy metals. Another possible modification would be the insertion of multiple Sp1-binding sites. Moreover, the promoter sequences may be combined with regulatory sequences which allow the transcription activity to be controlled or regulated. In this way the promoter can be made repressible or inducible. This may be achieved, for example, by linking with sequences which constitute binding sites for transcription factors with a positive or negative regulating effect. The above-mentioned

transcription factor SP-1, for example, has a positive influence on transcription activity. Another example is the binding site for activator protein AP-1 which can influence transcription both positively and negatively. The activity of AP-1 can be controlled by all kinds of factors, such as growth factors, cytokines and serum (Faisst and Meyer, 1992 and references therein). The transcription efficiency can also be increased by changing the promoter sequence by mutation (substitution, insertion, deletion) of one, two, three or more bases and then carrying out measurements in the CAT-test according to Example 4 to see whether the promoter activity is increased in this way. By adopting the measures described in this paragraph it is possible to achieve an optimum expression cassette which is of considerable use in the expression of heterologous gene products, especially in CHO-cells. The invention therefore also relates to an expression cassette obtained by one or more of these measures.

BSPR:

DNaseI-footprint and mutation analyses can be used to investigate which factors influence expression and whether the promoter activity can be further increased by deleting any negative control elements which may be present and by inserting other positive control elements. Investigations by other working groups have also shown that the expression of the Ub/S27a-gene can quite obviously be regulated by various factors. Thus, the group working with Shimbara showed that, in the terminal in vitro differentiation of human leukaemia cell lines (HL-60, K562, U937, THO1), the expression of the Ub/S27a-gene is suppressed by the addition of various substances such as TPA (12-O-tetra-decanoylphosphol-13-acetate), DMSO, retinoic acid and 1,25-dihydroxy vitamin D3 into the culture medium (Shimbara et al., 1993). Moreover, the group working with Wong established over-expression of the Ub/S27a-gene in carcinoma cells of the large intestine (Wong et al., 1993). The gene expression correlated with the clinical tumour stages with higher expression in more advanced cancer.

11. Document ID: US 6004805 A

L9: Entry 11 of 23

File: USPT
Dec 21, 1999

US-PAT-NO: 6004805
DOCUMENT-IDENTIFIER: US 6004805 A
TITLE: Transcriptional enhancer from milk protein genes
DATE-ISSUED: December 21, 1999

US-CL-CURRENT: 435/325; 435/243, 435/320.1, 435/410, 536/24.1

APPL-NO: 7/ 891541
DATE FILED: May 29, 1992

IN: Casperson; Gerald F., Schmidhauser; Christian T., Bissell; Mina J.

AB: The invention relates to novel enhancer nucleotide sequences which stimulate transcription of heterologous DNA in cells in culture. The enhancers are derived from major

milk protein genes by the process of deletion mapping and functional analysis. The invention also relates to expression vectors containing the novel enhancers.

File: USPT

Oct 26, 1999

L9: Entry 11 of 23

File: USPT

Dec 21, 1999

DOCUMENT-IDENTIFIER: US 6004805 A

TITLE: Transcriptional enhancer from milk protein genes

BSPR:

Schmitt-Ney, et al., (1991) identified binding sites for five nuclear proteins within the rat .beta.-casein promoter. Four were found in HC11 cell nuclear extracts. Two of the HC11 activities increased (activities C & D) and two decreased (activities A & B) following hormonal induction of casein expression. The A & B activities are thought to mediate repression because mutations affecting A binding caused an increase in basal (uninduced) promoter activity. The fifth binding activity, termed MGF, was found only in pregnant and lactating mammary gland (but not in HC11 cells). MGF binds to two sites, one between -80 and -100 and the other between -130 and -150. These sequences are conserved in other casein genes and in casein genes of other species. Mutations in the MGF binding sites that decrease protein binding also decrease transcriptional activity (Schmitt-Ney, et al., 1991, 1992).

12. Document ID: US 5972643 A

L9: Entry 12 of 23

File: USPT

Oct 26, 1999

US-PAT-NO: 5972643

DOCUMENT-IDENTIFIER: US 5972643 A

TITLE: Isolated polynucleotide molecules encoding CTCF, a CCCTC-binding factor
DATE-ISSUED: October 26, 1999

US-CL-CURRENT: 435/69.1; 435/243, 435/252.33, 435/320.1, 435/325, 536/23.5

APPL-NO: 8/ 475844

DATE FILED: June 7, 1995

PARENT-CASE:

RELATED APPLICATIONS This application is a continuation-in-part of Ser. No. 08/261,680, filed Jun 17, 1994 now abandoned.

IN: Lobanenkov; Victor L., Neiman; Paul E., Klenova; Elena M., Goodwin; Graham H., Filippova; Galina N., Collins; Steven J., Nicolas; Robert H.

AB: Polynucleotide molecules encoding CTCF are isolated and purified and sequenced. The CTCF proteins and antibodies thereto can be used to identify mutant CTCFs in methods of diagnosis.

L9: Entry 12 of 23

DOCUMENT-IDENTIFIER: US 5972643 A

TITLE: Isolated polynucleotide molecules encoding CTCF, a CCCTC-binding factor

DEPR:

The vectors were transfected essentially as described in Example 2, and polyclonal stably transfected cell lines were established by pooling all G418-resistant clones from each transfection. In stable transfection experiments, CAT activity, normalized to the internal copy number control .beta.-galactosidase activity, was assayed in cell extracts prepared from an equal number of transfected cells as described (Seed and Sheen, Gene 67: 271-277, 1988). Since transcription of the endogenous c-myc gene is dependant on cell growth conditions (for review see Marcu et al., Ann. Rev. Biochem. 61: 809-60, 1992), the contribution of CTCF to the regulation of the promoter activity might also be dependant on the proliferation status of stably transfected cells. Therefore, CAT activity was measured in cells grown under three different conditions: (1) normal growth, when cells were passaged every third day and did not reach confluence; (2) growth arrest, when confluent cells were kept in serum deprived media for 2.5 days; (3) serum response, when confluent cells were serum-starved for 2 days and then transferred to a fresh serum containing media for 12 hours prior to harvest. Under all three different cell growth conditions, pAPacaCAT transfectants (the "ACA" mutation in pAPacaCAT eliminated CTCF binding to the +30 site of the P2 promoter) had 3- to 6-fold increase reporter gene transcription activity suggesting that CTCF binding inhibits P2 promoter activity. The repressing effect of CTCF binding to the P2-proximal site (the wild-type c-myc promoter in pAPwtCAT) appeared most profound in growth-arrested cells (i.e. under conditions when transcription from the c-myc promoter was reported to be inhibited (Kelly and Siebenlist, Ann. Rev. Immunol. 4: 317-338, 1986)). Thus, mutational analysis of the P2-proximal CTCF-binding site strongly suggested that CTCF is a repressor of transcription from the major human c-myc gene promoter.

13. Document ID: US 5959094 A ✓

L9: Entry 13 of 23

File: USPT

Sep 28, 1999

US-PAT-NO: 5959094

DOCUMENT-IDENTIFIER: US 5959094 A

TITLE: p75 TNF receptor promoters
DATE-ISSUED: September 28, 1999

US-CL-CURRENT: 536/24.1

APPL-NO: 8/ 737371

DATE FILED: November 12, 1996

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS This application is a 371 of PCT/US95/05853, filed May 11, 1995.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

	APPL-NO	APPL-DATE
IL	109633	May 11, 1994

PCT-DATA:
APPL-NO

	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/US95/05853	May 11, 1995	WO95/31206	Nov 23, 1995	Nov 12, 1996	Nov 12, 1996

IN: Wallach; David, Kuhnert; Peter, Ehrhardt; Gotz, Kemper; Oliver

AB: The present invention is an isolated DNA molecule comprising a promoter of the human p75 TNF-R gene having a sequence consisting of a 5' upstream promoter sequence contained within an approximately 2.0 kbp NcoI fragment of a genomic clone of human p75 TNF-R, or an intron promoter sequence located in the first intron between the first and second exons and contained within an approximately 1.9 kbp SmaI fragment of a genomic clone of human p75 TNF-R. A composition comprising the DNA molecule and a carrier is also described.

L9: Entry 13 of 23

File: USPT

Sep 28, 1999

DOCUMENT-IDENTIFIER: US 5959094 A
TITLE: p75 TNF receptor promoters

DEPR:

Functional motifs in the promoter region (i.e. 5' upstream and first intron promoters) and the inhibitory region present in the first intron region upstream of the intron promoter (see Example 3) can be identified by step-wise deletion of nucleic acid sequence from the 3' and/or 5' end of the promoter and/or inhibitory regions by conventional means (Erase-a-Base kit, Promega Corp.). The deleted promoter or inhibitory region fragments are then tested for activity. Likewise, internal sequences can be deleted or changed by in vitro mutagenesis or linker scanning (31). Motifs that bind activating transcription factors are revealed by a loss of promoter activity when deleted or mutated. Conversely, motifs that bind transcription factors which suppress promoter activity are identified by mutated or deleted promoter fragments which have increased activity, compared to the wild-type promoter. Likewise, motifs concerned with activation or inhibition of the transcription inhibitory region can also be identified, which bind inhibitory factors or inhibition suppressor factors. A detailed analysis of these motifs is then carried out by chemical synthesis of oligonucleotides with the sequence of the original motif, and mutated forms of it. These oligonucleotides are linked to the promoter fragments lacking the

corresponding motifs, and the resulting construct is tested for promoter activity. If the original activity is restored, the motif can be regarded as functionally unchanged, i.e. those mutations that have been introduced into the motif, do not interfere with its function. On the other hand, if less promoter activity is observed with a mutated motif, it can be concluded that the nucleotides which were changed compared to the wild-type motif, are essential for its function.

14. Document ID: US 5914245 A

L9: Entry 14 of 23

File: USPT

Jun 22, 1999

US-PAT-NO: 5914245
DOCUMENT-IDENTIFIER: US 5914245 A
TITLE: Solid phase enzyme kinetics screening in microcolonies
DATE-ISSUED: June 22, 1999

US-CL-CURRENT: 435/19; 422/50, 435/14, 435/15, 435/23, 435/24, 435/25, 435/283.1, 435/4, 435/808, 435/968

APPL-NO: 9/ 098202
DATE FILED: June 16, 1998

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application No. 60/082,440, filed Apr. 20, 1998.

IN: Bylina; Edward J., Coleman; William J., Dilworth; Michael R., Silva; Christopher M., Yang; Mary M., Youvan; Douglas C.

AB: A MicroColonyImager instrument and solid phase methods to screen cells expressing mutagenized enzymes for enhanced activity. The MicroColonyImager instrument and methods permit high throughput screening of enzyme libraries by time course analyses of single-pixels, using either absorption, fluorescence or FRET. This high throughput assay can detect small differences in enzyme rates within microcolonies grown at a nearly confluent density on an assay disk. Each microcolony is analyzed simultaneously at single-pixel resolution, requiring less than 100 nl substrate/measurement. By simultaneously assaying different substrates tagged with spectrally distinct chromogenic or fluorogenic reporters, the substrate specificity of an enzyme can be changed.

L9: Entry 14 of 23

File: USPT

Jun 22, 1999

DOCUMENT-IDENTIFIER: US 5914245 A
TITLE: Solid phase enzyme kinetics screening in microcolonies

DEPR:

Usually, pools of potentially useful bacterial mutants are analyzed by growing up small aliquots of each mutant clone and rupturing the cells in a French press. The enzyme

activity of each of these crude lysates can then be analyzed spectrophotometrically by adding a small volume of the crude lysate to a solution of an optical signal substrate, e.g., p-nitrophenyl derivatized substrate, which releases the bright yellow p-nitrophenol, or PNP, upon hydrolysis. This technique cannot provide information on the specific activity (k.sub.cat), however, because the protein concentration of the enzyme of interest is not known. Knowledge of the specific activity is important because apparent increases in velocity could simply be the result of increased synthesis of the protein (i.e., promoter-up mutations). Since there are a multitude of proteins in the lysate, the mutagenized enzyme must first be purified before a measurement of k.sub.cat can be made. For a candidate pool of many potential mutants, this is a laborious and time-consuming process.

15. Document ID: US 5847102 A ✓

L9: Entry 15 of 23

File: USPT

Dec 8, 1998

US-PAT-NO: 5847102
DOCUMENT-IDENTIFIER: US 5847102 A
TITLE: Cold induced promoter from winter Brassica napus
DATE-ISSUED: December 8, 1998

US-CL-CURRENT: 536/24.1; 435/320.1, 435/91.41, 536/23.6

APPL-NO: 8/ 421044
DATE FILED: April 12, 1995

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

	APPL-NO	APPL-DATE
CA	2146712	April 10, 1995

IN: Singh; Jas, White; Theresa Catherine, Jiang; Chao

AB: A 1.2 kb fragment of the 5' regulatory region (from bp -1107 to +100) was fused to the GUS (.beta.-glucuronidase) reporter gene and BN115-promoted GUS expression was observed in green tissues of transgenic Brassica napus plants only after incubation at 2.degree. C. No expression was observed after incubation at 22.degree. C., either in the presence or absence of abscisic acid. Microprojectile bombardment of winter B. napus leaves with a BN115 promoter/GUS construct yielded similar results and was used to analyze a series of deletions from the 5' end of the promoter. Results obtained from transient expression studies showed that the low temperature regulation of BN115 expression involves a possible enhancer region between bp -1107 and -606 and a second positive regulatory region located between bp -302 and -274. Deletion analyses and results from replacement with a truncated CaMV (cauliflower mosaic virus) 35S promoter suggest that the minimal size required for any maintenance of low temperature GUS expression is a -300 bp fragment. Within this fragment are two 8 bp

elements with the sequence

TGGCCGAC, which are identical to those present in the positive regulatory region of the promoter of the homologous Arabidopsis cor15a gene and to a 5 bp core sequence in the low temperature- and dehydration-responsive elements (LTREs and DREs) identified in the promoter regions of several cold-responsive Arabidopsis thaliana genes. Mutation of either one or both of the GGCAC core sequence of the putative LTRE's to AATTC resulted in loss of cold-inducible gene expression, providing for the first time, direct evidence that CCGAC is required for low temperature expression. Furthermore, replacement of an enhancer region (-605 to -1107) of the promoter with a more active enhancer from the 35S constitutive cauliflower mosaic virus (CaMV) promoter resulted in a "hybrid" promoter with increased low temperature induced activity several fold over that of the native promoter.

L9: Entry 15 of 23

File: USPT

Dec 8, 1998

DOCUMENT-IDENTIFIER: US 5847102 A
TITLE: Cold induced promoter from winter Brassica napus

ABPL:

A 1.2 kb fragment of the 5' regulatory region (from bp -1107 to +100) was fused to the GUS (.beta.-glucuronidase) reporter gene and BN115-promoted GUS expression was observed in green tissues of transgenic Brassica napus plants only after incubation at 2.degree. C. No expression was observed after incubation at 22.degree. C., either in the presence or absence of abscisic acid. Microprojectile bombardment of winter B. napus leaves with a BN115 promoter/GUS construct yielded similar results and was used to analyze a series of deletions from the 5' end of the promoter. Results obtained from transient expression studies showed that the low temperature regulation of BN115 expression involves a possible enhancer region between bp -1107 and -606 and a second positive regulatory region located between bp -302 and -274. Deletion analyses and results from replacement with a truncated CaMV (cauliflower mosaic virus) 35S promoter suggest that the minimal size required for any maintenance of low temperature GUS expression is a -300 bp fragment. Within this fragment are two 8 bp elements with the sequence TGGCCGAC, which are identical to those present in the positive regulatory region of the promoter of the homologous Arabidopsis cor15a gene and to a 5 bp core sequence in the low temperature- and dehydration-responsive elements (LTREs and DREs) identified in the promoter regions of several cold-responsive Arabidopsis thaliana genes. Mutation of either one or both of the GGCAC core sequence of the putative LTRE's to AATTC resulted in loss of cold-inducible gene expression, providing for the first time, direct evidence that CCGAC is required for low temperature expression. Furthermore, replacement of an enhancer region (-605 to -1107) of the promoter with a more active enhancer from the 35S constitutive cauliflower mosaic virus (CaMV) promoter resulted in a "hybrid" promoter with increased low temperature induced activity several fold over that of the native promoter.

16. Document ID: US 5795975 A

L9: Entry 16 of 23

File: USPT

Aug 18, 1998

US-PAT-NO: 5795975
DOCUMENT-IDENTIFIER: US 5795975 A
TITLE: TNF receptor promoter
DATE-ISSUED: August 18, 1998

US-CL-CURRENT: 536/24.1; 435/320.1

APPL-NO: 8/ 532309
DATE FILED: September 22, 1995

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS The present application is a continuation-in-part of U.S. Ser. No. 08/178,564 filed Jan. 7, 1994, abandoned the entire contents of which are hereby incorporated by reference.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

	APPL-NO	APPL-DATE
IL	104355	January 10, 1993

IN: Wallach; David, Kemper; Oliver, Kuhnert; Peter

AB: A DNA molecule containing the endogenous first intron-located p55 TNF-R gene promoter/enhancer sequence is provided. Also provided is a DNA molecule which contains a gene in operative association with a promoter sequence that includes the endogenous first intron-located p55 TNF-R gene promoter/enhancer sequence.

L9: Entry 16 of 23

File: USPT

Aug 18, 1998

DOCUMENT-IDENTIFIER: US 5795975 A
TITLE: TNF receptor promoter

DEPR:
Functional transcription factor-binding motifs in the promoter region can be identified by step-wise deletion of nucleic acid sequence from the 3' and/or 5' end of the promoter by conventional means (Erase-a-Base kit, Promega Corp.). The deleted promoter fragments are then tested for activity. Likewise, internal sequences can be deleted or changed by in vitro mutagenesis or linker scanning (37). Motifs that bind activating transcription factors are revealed by a loss of promoter activity when deleted or mutated. Conversely, motifs that bind transcription factors which suppress promoter activity are identified by mutated or deleted promoter fragments which have increased activity, compared to the wild-type promoter. A detailed analysis of these motifs is then carried out by chemical synthesis of oligonucleotides with the sequence of the original motif, and mutated forms of it. These oligonucleotides are linked to the promoter fragments lacking the corresponding motifs, and the resulting construct is tested for promoter activity. If the original activity is restored,

the motif can be regarded as functionally unchanged, i.e., those mutations that were introduced into the motif, do not interfere with its function. On the other hand, if less promoter activity is observed with a mutated motif, it can be concluded that the nucleotides which were changed compared to the wild-type motif, are essential for its function.

17. Document ID: US 5643792 A

L9: Entry 17 of 23

File: USPT

Jul 1, 1997

US-PAT-NO: 5643792
DOCUMENT-IDENTIFIER: US 5643792 A
TITLE: Mutant strain of Pichia pastoris which utilizes methanol in the presence of glucose
DATE-ISSUED: July 1, 1997

US-CL-CURRENT: 435/254.23; 536/24.1

APPL-NO: 8/ 181242
DATE FILED: January 13, 1994

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

	APPL-NO	APPL-DATE
JP	5-004289	January 13, 1993

IN: Okabayashi; Ken, Ohmura; Takao, Yokoyama; Kazumasa, Kawabe; Haruhide

AB: A methylotrophic and glucotrophic mutant strain capable of producing a heterologous protein and a method for producing a heterologous protein, comprising culture of the mutant strain. The mutant strain of the present invention can be grown in a medium containing both methanol and glucose, with the effect that the growth of the strain and production of a heterologous protein proceed at the same time. Accordingly, a heterologous protein can be produced in a large amount in a short time.

L9: Entry 17 of 23

File: USPT

Jul 1, 1997

DOCUMENT-IDENTIFIER: US 5643792 A
TITLE: Mutant strain of Pichia pastoris which utilizes methanol in the presence of glucose

BSPR:
It has now been found that the mutation of the AOX2 promoter of the strain results in an enhanced promoter activity to afford an increased production of AOX by the AOX2 gene. Thus, the mutation of the AOX2 promoter has made it possible for the strain to grow well in a medium containing methanol.

18. Document ID: US 5627033 A

L9: Entry 18 of 23

File: USPT

May 6, 1997

US-PAT-NO: 5627033
DOCUMENT-IDENTIFIER: US 5627033 A
TITLE: Mammalian expression vectors
DATE-ISSUED: May 6, 1997

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/358, 435/365,
435/461, 435/91.41

APPL-NO: 8/ 411490
DATE FILED: March 28, 1995

IN: Smith; John M., Humphrey; John E., Tsang; Monica L.,
Weatherbee; James A.

AB: A vector system that allows the rapid and effective screening of
recombinant
constructs. The vector system includes a marker protein useful for
identifying transfected cell
lines, wherein the promoter used to express the marker protein has be
substantially weakened in
comparison to its corresponding wild type form.

L9: Entry 18 of 23

File: USPT

May 6, 1997

DOCUMENT-IDENTIFIER: US 5627033 A
TITLE: Mammalian expression vectors

BSPR:
Pauly et al. further describe a method for the determination of relative and
absolute promoter
activity levels that is useful in the present invention. In particular, the
method of Pauly et al.
can be used to compare the activity of mutated promoter regions with their
corresponding wild type
forms.

19. Document ID: US 5607672 A

L9: Entry 19 of 23

File: USPT

Mar 4, 1997

US-PAT-NO: 5607672
DOCUMENT-IDENTIFIER: US 5607672 A
TITLE: Replacement therapy for dental caries
DATE-ISSUED: March 4, 1997

US-CL-CURRENT: 424/50; 424/93.44, 435/252.3

APPL-NO: 8/ 486037
DATE FILED: June 7, 1995

IN: Hillman; Jeffrey D.

AB: Recombinant Streptococcus mutans strains characterized by a
deficiency in lactic
acid production and production of a recombinant alcohol dehydrogenase
(ADH) are described,

These recombinant S. mutans strains are suitable for use in a method for
preventing treating
dental caries.

L9: Entry 19 of 23

File: USPT

Mar 4, 1997

DOCUMENT-IDENTIFIER: US 5607672 A
TITLE: Replacement therapy for dental caries

DEPR:
Methods for producing variants using recombinant techniques are well
known in the art (see, for
example, Sambrook et al., 1989, supra). For example, S. mutans JH1000
variants having increased
bacteriocin activity can be generated by expression of multiple copies of
bacteriocin-encoding DNA,
and/or mutation of the bacteriocin promoter to provide higher levels of
transcription.

20. Document ID: US 5260196 A

L9: Entry 20 of 23

File: USPT

Nov 9, 1993

US-PAT-NO: 5260196
DOCUMENT-IDENTIFIER: US 5260196 A
TITLE: Method for the diagnosis of Tourette syndrome and associated
disorders

DATE-ISSUED: November 9, 1993

US-CL-CURRENT: 435/25; 424/94.4

APPL-NO: 7/ 715660
DATE FILED: June 14, 1991

PARENT-CASE:
This application is a continuation-in-part of each of copending application
Ser. No. 125,577 (now
abandoned) filed Nov. 25, 1987, copending application Ser. No. 271,653
filed Nov. 16, 1988,
copending application Ser. No. 410,831 (now abandoned) filed Sep. 22,
1989, and copending
application Ser. No. 562,596 filed Aug. 3, 1990.

IN: Comings; David E.

AB: Differences in the rate of kynurenine formation in the lysates of
red blood cells of
a patient suspected of having Tourette syndrome, Tourette syndrome
associated disorders or
Tourette spectrum disorders and of a control are used for the diagnosis of
such disorders.

L9: Entry 20 of 23

File: USPT

Nov 9, 1993

DOCUMENT-IDENTIFIER: US 5260196 A

TITLE: Method for the diagnosis of Tourette syndrome and associated disorders

BSPR:

It is anticipated that the mutations causing hyperinducability or increased activity of the

TD02-ID02 genes occur in or near the promoter regions of the 5' regulatory sequences of the

TD02-ID02 genes in or near glucocorticoid response elements (GRE) or other induction response

elements, or in the expressed-translated portions of the gene, affecting

heme, tryptophan, copper or

O2 or O-binding at or near or the active catalytic site.

21. Document ID: US 5847102 A

L9: Entry 21 of 23

File: EPAB

Dec 8, 1998

PUB-NO: US005847102A

DOCUMENT-IDENTIFIER: US 5847102 A

TITLE: Cold induced promoter from winter Brassica napus

PUBN-DATE: December 8, 1998

INT-CL (IPC): C12N 15/29; C12N 15/82; C12N 15/11; A01H 1/00

EUR-CL (EPC): C07K014/415; C12N015/82

APPL-NO: US42104495

APPL-DATE: April 12, 1995

PRIORITY-DATA: CA02146712A (April 10, 1995)

IN: SINGH, JAS, WHITE, THERESA CATHERINE, JIANG, CHAO

AB: A 1.2 kb fragment of the 5' regulatory region (from bp -1107 to +100) was fused to the GUS (beta -glucuronidase) reporter gene and BN115-promoted GUS expression was observed in green tissues of transgenic Brassica napus plants only after incubation at 2 DEG C. No

expression was observed after incubation at 22 DEG C., either in the presence or absence of

abscisic acid. Microprojectile bombardment of winter B. napus leaves with a BN115 promoter/GUS

construct yielded similar results and was used to analyze a series of deletions from the 5' end

of the promoter. Results obtained from transient expression studies showed that the low

temperature regulation of BN115 expression involves a possible enhancer region between bp -1107

and -606 and a second positive regulatory region located between bp -302 and -274. Deletion

analyses and results from replacement with a truncated CaMV (cauliflower mosaic virus) 35S

promoter suggest that the minimal size required for any maintenance of low temperature GUS

expression is a -300 bp fragment. Within this fragment are two 8 bp elements with the sequence

TGGCCGAC, which are identical to those present in the positive regulatory region of the

promoter of the homologous Arabidopsis cor15a gene and to a 5 bp core sequence in the low

temperature- and dehydration-responsive elements (LTREs and DREs) identified in the promoter

regions of several cold-responsive Arabidopsis thaliana genes. Mutation

of either one or both

of the GGCAC core sequence of the putative LTRE's to AATTC resulted in loss of cold-inducible

gene expression, providing for the first time, direct evidence that CCGAC is required for low

temperature expression. Furthermore, replacement of an enhancer region (-605 to -1107) of the

promoter with a more active enhancer from the 35S constitutive cauliflower mosaic virus (CaMV)

promoter resulted in a "hybrid" promoter with increased low temperature induced activity

several fold over that of the native promoter.

L9: Entry 21 of 23

File: EPAB

Dec 8, 1998

DOCUMENT-IDENTIFIER: US 5847102 A

TITLE: Cold induced promoter from winter Brassica napus

FPAR:

A 1.2 kb fragment of the 5' regulatory region (from bp -1107 to +100) was fused to the GUS (beta

-glucuronidase) reporter gene and BN115-promoted GUS expression was observed in green tissues of

transgenic Brassica napus plants only after incubation at 2 DEG C. No expression was observed after

incubation at 22 DEG C., either in the presence or absence of abscisic acid. Microprojectile

bombardment of winter B. napus leaves with a BN115 promoter/GUS construct yielded similar results

and was used to analyze a series of deletions from the 5' end of the promoter. Results obtained from

transient expression studies showed that the low temperature regulation of BN115 expression involves

a possible enhancer region between bp -1107 and -606 and a second positive regulatory region located

between bp -302 and -274. Deletion analyses and results from replacement with a truncated CaMV

(cauliflower mosaic virus) 35S promoter suggest that the minimal size required for any maintenance

of low temperature GUS expression is a -300 bp fragment. Within this fragment are two 8 bp elements

with the sequence TGGCCGAC, which are identical to those present in the positive regulatory region

of the promoter of the homologous Arabidopsis cor15a gene and to a 5 bp core sequence in the low

temperature- and dehydration-responsive elements (LTREs and DREs) identified in the promoter regions

of several cold-responsive Arabidopsis thaliana genes. Mutation of either one or both of the GGCAC

core sequence of the putative LTRE's to AATTC resulted in loss of cold-inducible gene expression,

providing for the first time, direct evidence that CCGAC is required for low temperature expression.

Furthermore, replacement of an enhancer region (-605 to -1107) of the promoter with a more active

enhancer from the 35S constitutive cauliflower mosaic virus (CaMV) promoter resulted in a "hybrid"

promoter with increased low temperature induced activity several fold over that of the native

promoter.

22. Document ID: AU 9948037 A, WO 200102572 A1

L9: Entry 22 of 23

File: DWPI

Jan 22, 2001

DERWENT-ACC-NO: 2001-080986
DERWENT-WEEK: 200125
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TITLE: Polynucleotide encoding an Arabidopsis thaliana flowering time associated (FWA) protein from the vernalisation pathway, useful for genetically altering the flowering time characteristics, i.e.

delaying or accelerating flowering, of a plant

PRIORITY-DATA: 1999WO-NL00414 (July 2, 1999)

PATENT-FAMILY:

PUB-NO

PUB-DATE

LANGUAGE

PAGES

MAIN-IPC

AU 9948037 A
January 22, 2001
000
C12N015/29

WO 200102572 A1
January 11, 2001
E
040
C12N015/29

APPLICATION-DATA:

PUB-NO

APPL-DATE

APPL-NO

DESCRIPTOR

AU 9948037A
July 2, 1999
1999AU-0048037

AU 9948037A
July 2, 1999
1999WO-NL00414

AU 9948037A
WO 200102572
Based on

WO 200102572A1
July 2, 1999
1999WO-NL00414

INT-CL (IPC): A01H 5/00; C07K 14/415; C12N 5/10; C12N 15/29; C12N 15/82

IN: KOORNNEEF, M, PEETERS, A J M, SOPPE, W J J

AB: NOVELTY - A polynucleotide (I) encoding an Arabidopsis thaliana flowering time associated (FWA) protein from the vernalisation pathway, is new. N1 encodes a FWA amino acid sequence (S1, S2, S3 or S4), S1 and S2 are sequences encoded by nucleic acids N1 and N2, respectively. The sequence of N1, N2, S3 and S4 are defined in the specification., DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:, (1) a nucleic acid sequence (II) encoding a fragment of S1, S2, S3 or S4, where the fragment is at least 4 amino acids in length;, (2) a nucleic acid (III) sequence capable of hybridizing under stringent to moderate conditions with the N1 or N2;, (3) a mutant (IV) of (I), (II) or (III), where the mutation is such that the expression product of the nucleic acid sequence exhibits reduced FWA activity in comparison to the expression product of the equivalent non-mutant nucleic acid sequence, when the sequences are operatively linked to the identical promoters;, (4) a nucleic acid sequence (V) of (I), (II), (III) or (IV) operatively linked to an expression regulating

sequence not normally associated with the FWA gene;, (5) a vector comprising (I), (II), (III), (IV) or (V), where the vector is constructed such that it is suitable for transferring the nucleic acid to a plant, plant cell or plant part;, (6) a plant, plant part or plant cell into which a nucleic acid sequence of (I), (II), (III), (IV) or (V) or the vector of (5) has been introduced;, (7) a genetically engineered plant, plant cell or plant part having an altered expression of the FWA gene in comparison to that of the non-genetically engineered starting material under equivalent conditions;, (8) plant, plant part or plant cell other than Arabidopsis thaliana comprising a nucleic acid sequence of (I), (II), (III), (IV) or (V) or the vector of (5);, (9) plant, plant part or plant cell comprising an antisense sequence of a nucleic acid sequence of (I), (II), (III), (IV) or (V) or the vector of (5);, (10) a method of amending the flowering time characteristics of a plant, comprising genetically altering the expression level of the FWA gene by introducing a nucleic acid sequence of (I), (II), (III), (IV) or (V) or the vector of (5) into a plant, plant cell or plant part, where the cell or plant part is suitable for cultivation to a plant;, (11) a method of lengthening the flowering time of a plant by reducing the level of expression of FWA by genetic manipulation of the FWA gene i.e. genetic manipulation of the encoding sequence or the expression regulating sequence of FWA gene;, (12) a method of lengthening the flowering time of a plant by reducing the level of expression of FWA by introducing sense or antisense copies of nucleic acid sequences capable of exhibiting co-suppression or gene silencing of the FWA gene in cells of the plant, where the sequences are (I), (II), (III), (IV) or (V), or the vector of (5);, (13) a method (M1) of selectively shortening or lengthening the flowering time of a plant by introducing an inducible expression regulating sequence other than the expression regulating sequence normally associated with FWA gene via genetic engineering; and, (14) a method (M2) of producing flowering of a recombinant plant at a time different to that of the corresponding non-recombinant plant under the same environmental conditions, comprising cultivation of a plant, plant part or plant cell of (6), (7), (8) or (9);, USE - The nucleic acid sequences and vectors are used for genetically altering the flowering time characteristics, i.e. delaying or accelerating flowering, of a plant by introducing, in a sense or antisense direction, any of the sequences into the plant, plant part or a plant cell. The nucleic acid sequences are also used for detecting and optionally isolating FWA genes (all claimed).

L9: Entry 22 of 23

File: DWPI

Jan 22, 2001

DERWENT-ACC-NO: 2001-080986
DERWENT-WEEK: 200125
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TITLE: Polynucleotide encoding an Arabidopsis thaliana flowering time associated (FWA) protein from the vernalisation pathway, useful for genetically altering the flowering time characteristics, i.e. delaying or accelerating flowering, of a plant

ABTX:

(3) a mutant (IV) of (I), (II) or (III), where the mutation is such that the

expression product of
the nucleic acid sequence exhibits reduced FWA activity in comparison to
the expression product of
the equivalent non-mutant nucleic acid sequence, when the sequences are
operatively linked to the
identical promoters;

23. Document ID: JP 3124289 B2, EP 444759 A, WO 9113154 A,
FI 9100975 A, PT 96913 A, JP 05500456 W, US
5246838 A, EP 444759 B1, DE 69131112 E

L9: Entry 23 of 23

File: DWPI

Jan 15, 2001

DERWENT-ACC-NO: 1991-261526
DERWENT-WEEK: 200106
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TITLE: DNA sequence encoding signal peptidase - for cloning and
over-expression of leader peptidase
gene for enhanced rate of protein processing

PRIORITY-DATA: 1991EP-0200431 (February 28, 1991),
1990EP-0200477 (February 28, 1990),
1990EP-0203509 (December 24, 1990)

PATENT-FAMILY:
PUB-NO

PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 3124289 B2 January 15, 2001		038	C12N015/09
EP 444759 A September 4, 1991		051	
WO 9113154 A September 5, 1991		000	
FI 9100975 A August 29, 1991		000	
PT 96913 A October 31, 1991		000	
JP 05500456 W February 4, 1993		025	C12N015/57
US 5246838 A September 21, 1993		039	C12P021/06
EP 444759 B1 April 14, 1999	E	000	C12N015/57
DE 69131112 E May 20, 1999		000	C12N015/57

APPLICATION-DATA:
PUB-NO

APPL-DATE	APPL-NO	DESCRIPTOR
JP 3124289B2 February 28, 1991	1991JP-0505870	
JP 3124289B2 February 28, 1991	1991WO-NL00033	
JP 3124289B2	JP 5500456	Previous Publ.
JP 3124289B2	WO 9113154	Based on
EP 444759A February 28, 1991	1991EP-0200431	
JP 05500456W February 28, 1991	1991JP-0505870	
JP 05500456W February 28, 1991	1991WO-NL00033	
JP 05500456W	WO 9113154	Based on
US 5246838A February 28, 1991	1991US-0662005	
EP 444759B1 February 28, 1991	1991EP-0200431	
DE 69131112E February 28, 1991	1991DE-0631112	
DE 69131112E February 28, 1991	1991EP-0200431	
DE 69131112E	EP 444759	Based on

INT-CL (IPC): C07H 21/04; C12N 1/21; C12N 9/52; C12N 9/56; C12N
15/09; C12N 15/57; C12N 15/67; C12P 21/02; C12P
21/06; C12N 9/56; C12R 1/19; C12N 15/09; C12R 1/125

IN: BRON, S, QUAX, W J, SMITH, H E, VANDIJL, J M, VAN
DIJL, J M, VENENIA, G

AB: New DNA sequence encoding a signal peptidase is isolated
from a microorganism other
than E.coli. Also claimed are (1) DNA sequence encoding a signal
peptidase from Bacillus
subtilis; (2) DNA sequence which hybridises to (1) and is not E.coli signal
peptidase-encoding
DNA under stringent condns. and show proteolytic activity; (3) selection
of a heterologous
signal peptidase encoding gene comprising digestion of chromosomal
DNA and cloning the
fragments into an expression vehicle, transforming a host cell which
contains a reporter
protein and using a suitable assay to select transformed clones; (4)
selection of heterologous
signal peptidase encoding gene comprising cloning digested chromosomal
DNA fragments into a
cloning vehicle transforming a host cell having its leader peptidase gene
under the control of
a regulatable promoter and selecting normally growing cells under
non-permissive condns.; (5)
use of cloned or mutated signal peptidase gene to obtain increased
processing activity of
hetero- or homologous periplasmic, outer membrane or secreted protein;
(6) recombinant host
contg. an expression vehicle contg. a cloned DNA encoding a signal
peptidase and an expression
vehicle encoding the desired recombinant periplasmic, outer membrane or

secreted protein; (7) recombinant host where the wild type or mutated leader peptidase gene is over expressed; (8) mutant signal peptidase with increase processing activity of a hetero- or homologous periplasmic outer membrane or secreted protein., USE - Over-expression of a leader peptidase gene in a suitable host leads to an enhanced rate of protein processing., New DNA sequence encoding a signal peptidase is isolated from a microorganism other than E.coli., Also claimed are (1) DNA sequence encoding a signal peptidase from *Bacillus subtilis*; (2) DNA sequence which hybridises to (1) and is not E.coli signal peptidase-encoding DNA under stringent condns. and show proteolytic activity; (3) selection of a heterologous signal peptidase encoding gene comprising digestion of chromosomal DNA and cloning the fragments into an expression vehicle, transforming a host cell which contains a reporter protein and using a suitable assay to select transformed clones; (4) selection of heterologous signal peptidase encoding gene comprising cloning digested chromosomal DNA fragments into a cloning vehicle transforming a host cell having its leader peptidase gene under the control of a regulatable promoter and selecting normally growing cells under non-permissive condns.; (5) use of cloned or mutated signal peptidase gene to obtain increased processing activity of hetero- or homologous periplasmic, outer membrane or secreted protein; (6) recombinant host contg. an expression vehicle contg. a cloned DNA encoding a signal peptidase and an expression vehicle encoding the desired recombinant periplasmic, outer membrane or secreted protein; (7) recombinant host where the wild type or mutated leader peptidase gene is over expressed; (8) mutant signal peptidase with increase processing activity of a hetero- or homologous periplasmic outer membrane or secreted protein., USE - Over-expression of a leader peptidase gene in a suitable host leads to an enhanced rate of protein processing., Nucleic acid (cDNA) that encodes the prodn. of a proteolytic signal peptidase (SPase type I) derived from a *Bacillus* strain (e.g. *Bacillus subtilis*), and plasmids and expression vectors contg. this DNA are new. Host cells have been transformed with these plasmids and vectors and then propagated to produce the exogenous enzyme. Further, host cells contg. a gene that produces a periplasmic outer membrane or secreted protein have been transformed with the Spase-I gene of *Salmonella typhimurium* or *Bacillus subtilis* and propagated to obtain increased protein yields. The nucleotide sequence of the cDNA and the amino acid sequence of the protein are given., USE - The process facilitates the microbiological prodn. of exogenous proteins.

sequence which hybridises to (1) and is not E.coli signal peptidase-encoding DNA under stringent condns. and show proteolytic activity; (3) selection of a heterologous signal peptidase encoding gene comprising digestion of chromosomal DNA and cloning the fragments into an expression vehicle, transforming a host cell which contains a reporter protein and using a suitable assay to select transformed clones; (4) selection of heterologous signal peptidase encoding gene comprising cloning digested chromosomal DNA fragments into a cloning vehicle transforming a host cell having its leader peptidase gene under the control of a regulatable promoter and selecting normally growing cells under non-permissive condns.; (5) use of cloned or mutated signal peptidase gene to obtain increased processing activity of hetero- or homologous periplasmic, outer membrane or secreted protein; (6) recombinant host contg. an expression vehicle contg. a cloned DNA encoding a signal peptidase and an expression vehicle encoding the desired recombinant periplasmic, outer membrane or secreted protein; (7) recombinant host where the wild type or mutated leader peptidase gene is over expressed; (8) mutant signal peptidase with increase processing activity of a hetero- or homologous periplasmic outer membrane or secreted protein.

ABEQ:

Also claimed are (1) DNA sequence encoding a signal peptidase from *Bacillus subtilis*; (2) DNA sequence which hybridises to (1) and is not E.coli signal peptidase-encoding DNA under stringent condns. and show proteolytic activity; (3) selection of a heterologous signal peptidase encoding gene comprising digestion of chromosomal DNA and cloning the fragments into an expression vehicle, transforming a host cell which contains a reporter protein and using a suitable assay to select transformed clones; (4) selection of heterologous signal peptidase encoding gene comprising cloning digested chromosomal DNA fragments into a cloning vehicle transforming a host cell having its leader peptidase gene under the control of a regulatable promoter and selecting normally growing cells under non-permissive condns.; (5) use of cloned or mutated signal peptidase gene to obtain increased processing activity of hetero- or homologous periplasmic, outer membrane or secreted protein; (6) recombinant host contg. an expression vehicle contg. a cloned DNA encoding a signal peptidase and an expression vehicle encoding the desired recombinant periplasmic, outer membrane or secreted protein; (7) recombinant host where the wild type or mutated leader peptidase gene is over expressed; (8) mutant signal peptidase with increase processing activity of a hetero- or homologous periplasmic outer membrane or secreted protein.

L9: Entry 23 of 23

File: DWPI

Jan 15, 2001

DERWENT-ACC-NO: 1991-261526
DERWENT-WEEK: 200106
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TITLE: DNA sequence encoding signal peptidase - for cloning and over-expression of leader peptidase gene for enhanced rate of protein processing

ABTX:

Also claimed are (1) DNA sequence encoding a signal peptidase from *Bacillus subtilis*; (2) DNA

Identification of Promoter Mutants Defective in Growth-Rate-Dependent Regulation of rRNA Transcription in *Escherichia coli*

RAMONA R. DICKSON,¹ TAMAS GAAL,² HERMAN A. DEBOER,³ PIETER L. DEHASETH,⁴
AND RICHARD L. GOURSE^{2*}

Department of Bacteriology, University of Wisconsin, 1550 Linden Drive, Madison, Wisconsin 53706²; Department of Genetics, University of Georgia, Athens, Georgia 30602¹; Gorlaeus Laboratories, Leiden University, 2300 AL Leiden, The Netherlands³; and Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106⁴

Received 5 April 1989/Accepted 31 May 1989

We measured the activities of 50 operon fusions from a collection of mutant and wild-type *rrnB* P1 (*rrnB*l_P in the nomenclature of B. J. Bachmann and K. B. Low [Microbiol. Rev. 44:1-56, 1980]) promoters under different nutritional conditions in order to analyze the DNA sequence determinants of growth rate-dependent regulation of rRNA transcription in *Escherichia coli*. Mutants which deviated from the wild-type -10 or -35 hexamers or from the wild-type 16-base-pair spacer length between the hexamers were unregulated, regardless of whether the mutations brought the promoters closer to the *E. coli* promoter consensus sequence and increased activity or whether the changes took the promoters further away from the consensus and reduced activity. These data suggest that rRNA promoters have evolved to maintain their regulatory abilities rather than to maximize promoter strength. Some double substitutions outside the consensus hexamers were almost completely unregulated, while single substitutions at several positions outside the -10 and -35 consensus hexamers exerted smaller but significant effects on regulation. These studies suggest roles for specific promoter sequences and/or structures in interactions with regulatory molecules and suggest experimental tests for models of rRNA regulation.

Ribosome synthesis rates in *Escherichia coli* are a direct function of rRNA transcription rates under most growth conditions (26, 34). In order to meet the cell's requirements for protein synthesis, cells transcribe rRNA and tRNA at approximately the square of the growth rate, a phenomenon termed growth rate-dependent regulation (29).

The mechanism responsible for growth rate control remains unclear. Previously, it was shown that a negative feedback system is responsible for rRNA and tRNA regulation (15-17, 28, 39) and that the system responds to the level of ribosomes that are capable of translation (10, 49). The magnitude of the signal made in response to the cell's translational capacity varies with the nutritional state of the culture. The identity of the signal is not known, although guanosine tetraphosphate (ppGpp) has been implicated as playing some role because of the virtually perfect inverse correlation between stable RNA synthesis and ppGpp concentration (36).

The target of the signal, whatever its identity, has been shown in at least three of the seven rRNA operons to be P1, the more upstream of the two rRNA promoters (*rrnB* and *rrnE* [15]; *rrnA* [37]). The DNA sequence determinants responsible for growth rate regulation are limited to positions between -4 and -50 with respect to the transcription start site (15). Experiments on two tRNA promoters, *tyrT* (46) and *leuV* (12), imply that the promoter sequence requirements for growth rate control of tRNA genes are limited to approximately the same regions. In addition to the regions required for growth rate regulation, upstream of the normal RNA polymerase-binding site in *rrnB* P1 (*rrnB*l_P in the nomenclature of Bachmann and Low [2b]), *tyrT*, and *leuV*,

there is a region called the upstream activation sequence (UAS), which is required for maximal promoter activity (4, 15, 23).

Mutations have been targeted to specific sites in the promoters *rrnB* P1 and *tyrT*, and activities resulting from the fusion of the mutant promoters to "reporter" genes have been measured under different growth conditions (15, 46). Such experiments have implicated DNA sequences required for growth rate-dependent control in the region between -20 and -50 (15) and in the region just downstream of the -10 hexamer (46). The mutations examined in both studies contained changes at multiple promoter positions.

We report here the examination of promoter mutants containing single substitutions, single deletions, single insertions, or multiple changes on growth rate control of an rRNA promoter. These studies were made possible by the construction by DNA synthesis of a large collection of nonselected variants of the *rrnB* P1 promoter, as reported in the accompanying paper (13). In that paper we showed that certain promoters exhibited relatively large differences in activity compared with the wild type when assayed as operon fusions to *lacZ* in lambda lysogens. We now show that in some cases these deviations result from or are accompanied by a loss of regulation, and we define positions and spacing in the promoter region important for control of rRNA synthesis.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phages. Construction of lambda lysogens containing *rrnB* P1 promoter mutations was described in the accompanying paper (13).

Growth rate dependence assays. β -Galactosidase assays were performed essentially as described in the accompanying

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ing paper (13), except cells were sonicated rather than treated with chloroform and sodium dodecyl sulfate because β -galactosidase recovery from permeabilized cells varies with growth conditions (47). Protein determinations were performed on samples of the same sonicated samples used for determining β -galactosidase activities, using the Bradford microassay technique (8) with the kit supplied by Bio-Rad Laboratories. Activities are expressed in Miller units per milligram of protein rather than in units per OD₆₀₀ unit, since cell size and therefore light-scattering measurements vary slightly at different growth rates. Three replicates of samples from each growth medium were done, with values generally within 15% of each other, and the average (illustrated as a single data point after scaling in Fig. 2; see below) was used to determine units per milligram of protein. In addition to the replicates done in each experiment, the data reported here on each strain contain the results of experiments performed on at least two separate days. The day-to-day variation is illustrated by the data points shown in Fig. 2. In each experiment, fusions to wild-type promoters were included as positive controls.

Different growth rates (expressed as μ [doublings per hour] at 30°C) in strain NK5031 were obtained by growing cells in LB or defined medium, which was AB medium (9) containing 0.005% thiamine, with the indicated supplements: AB, 0.4% glycerol, and 0.001% tryptophan (μ about 0.56 doublings per h); AB, 0.1% glycerol, 0.1% casamino acids, and 0.001% tryptophan (μ = 0.61); AB, 0.2% glucose, and 0.2% casamino acids (μ = 0.67); 90% AB, 0.2% glucose, 0.5% casamino acids, 0.001% tryptophan, and 10% LB (μ = 0.71); 50% LB and 50% AB (μ = 0.83); 100% LB (μ = 0.95); 80% AB, 0.1% glucose, 0.5% casamino acids, 0.001% tryptophan, and 20% LB (μ = 1.1). rRNA synthesis rates measured directly or with *rrnB* P1-*lacZ* fusions increase at approximately the square of the growth rate under these conditions (15, 29, 50). The host strain used, NK5031, was chosen because it exhibits easily recognizable plate phenotypes with small changes in *lacZ* expression (6, 31). The relatively narrow range in which the measurements were done was necessitated by the fact that the host strain chosen had a maximum growth rate of ≈ 1.2 at 30°C. The abscissa on the plots shown in Fig. 2 is therefore somewhat expanded, which tends to exaggerate minor day-to-day variations in growth rate.

Statistical analyses. The activity of each promoter (in β -galactosidase units per milligram of protein) was plotted versus μ , and a linear regression was performed (with the Statistix computer software package) in order to derive the equation for the resulting line. From this line, the activities of the various promoters at specific growth rates could be compared (Table 1). In order to make it possible to compare the slopes visually, the activities of all the promoters were then scaled to a value of 1.0 at a single growth rate of 0.9; the activities at all growth rates were then divided by the scaling factor and replotted, and a linear regression was performed on the transformed data. A graphic representation of the scaling procedure is shown in Fig. 1, and the transformed slopes for 28 of the mutants are illustrated in Fig. 2, drawn using the SigmaPlot computer graphics software package (Jandel Scientific). The slopes with standard errors were determined by computer analysis (Statistix). The standard errors of the slopes (Table 2) reflect both day-to-day variation and deviation from a linear fit. The ratio (R) of the mutant (M) to the wild-type (WT) slope (Table 2) with its standard error (SE) was computed by a propagation-of-

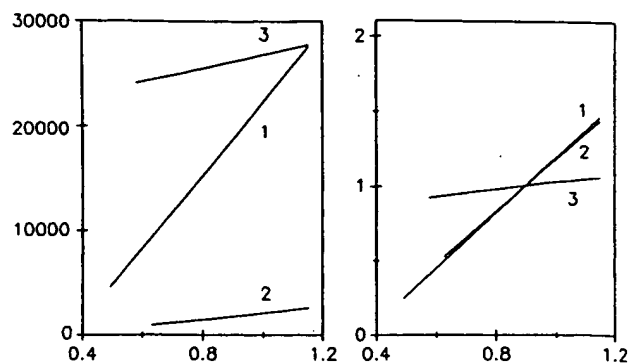


FIG. 1. Scaling procedure. (Left) Regulation of three fusions at different growth rates (untransformed data). The x-axis represents growth rate (μ [doublings per hour]), and the y-axis represents β -galactosidase activity in units per milligram of cell protein. Slopes: 1, wild-type promoter containing the UAS; 2, wild-type promoter lacking the UAS; 3, mutant promoter with higher than wild-type activity and defective regulation. (Right) Same data as in left panel, but the activities have been scaled to a value of 1 at a growth rate μ of 0.9. It can be seen that the wild-type promoters with and without the UAS were virtually identically regulated, while the mutant was unregulated.

errors calculation with the following equation (40): $SE\ R = R[(SE\ M\ slope/M\ slope)^2 + (SE\ WT\ slope/WT\ slope)^2]^{1/2}$.

There is no simple way to compare the regulatory capacities of different fusions, since the slopes of growth rate dependence curves from fusions with different promoter strengths cannot be compared directly. The method we used for comparing the regulation of the different promoters relies on scaling all the activities to the same value at a single growth rate, making it possible to compare the slopes visually and to derive a quantitative measure which we define as the fraction of regulation. Any promoter controlled exactly like the wild-type promoter will have a regulation value of 1.0, while a promoter whose activity increases only linearly with μ (horizontal line) will have a regulation value of 0, independent of the growth rate at which the scaling is performed. Promoters whose activities increase less than linearly with μ (negative slope) have a regulation value of less than 0, and promoters which increase in activity more steeply than the wild-type promoter have values of more than 1.0. For promoters differing from the wild type in regulation, we note that the growth rate at which the scaling procedure is performed slightly affects the absolute fraction obtained. The relative regulation of mutants with respect to each other, however, is independent of the growth rate at which the scaling is performed. We have arbitrarily chosen a growth rate of 0.9, in the middle of the range at which the regulation was measured, for scaling the activities of the different fusions.

Nomenclature of promoters. Wild-type promoters are designated by the extent of *rrnB* P1 sequence with respect to the transcription start site (e.g., [-88,+2]). Mutants are named as described in the accompanying paper (13).

Some mutant promoter constructions contain slightly different extents of *rrnB* P1 sequence. Therefore, three different wild-type constructions were used as controls, so that mutant promoters were always compared with wild-type promoters containing the same extents of *rrnB* sequence. There was slight variation in the growth rate dependence of the promoters used as controls. In the discussion which follows, mutant promoters which are designated as being unregulated exhibited much greater differences from the

TABLE 2. Regulation of mutant promoters

PROMOTER NAME	SEQUENCE LIMITS ^a	SLOPE (SCALED) ^b	MUTANT SLOPE/WT SLOPE ^c
WILD TYPE	-48,+1	1.49 (+/- 0.12)	
WILD TYPE	-50,+2	1.76 (+/- 0.12)	
WILD TYPE	-88,+2	1.86 (+/- 0.11)	
SUBSTITUTIONS			
C-1T,C-15G	-48,+1	0.16 (+/- 0.14)	0.11 (+/- 0.09)
A-3G,C-4T	"	1.54 (+/- 0.17)	1.03 (+/- 0.14)
C-4T	"	0.85 (+/- 0.11)	0.57 (+/- 0.08)
C-4T,T-9G	"	-6.77 (+/- 1.67)	-4.54 (+/- 1.18) ^e
C-5A	"	1.63 (+/- 0.13)	1.10 (+/- 0.13)
G-6T	"	0.90 (+/- 0.08)	0.60 (+/- 0.07)
G-8A,A-10T,A-24G	"	-1.17 (+/- 0.87)	-0.79 (+/- 0.59) ^e
T-12C	"	0.82 (+/- 0.24)	0.55 (+/- 0.17)
A-13G	"	-0.94 (+/- 0.56)	-0.63 (+/- 0.38)
T-14A	"	-1.16 (+/- 0.70)	-0.78 (+/- 0.47)
C-17T	"	0.84 (+/- 0.25)	0.56 (+/- 0.17)
C-17A,C-27A	"	0.78 (+/- 0.35)	0.52 (+/- 0.24)
T-18C	"	1.50 (+/- 0.17)	1.00 (+/- 0.14)
C-19T	"	1.79 (+/- 0.17)	1.20 (+/- 0.15)
C-19A,A-20C	"	0.23 (+/- 0.16)	0.15 (+/- 0.11)
A-20G	"	2.23 (+/- 0.23)	1.50 (+/- 0.20)
rac145 ^d	-153,+8	3.16 (+/- 0.28)	1.70 (+/- 0.18)
A-21T,T-22A	-50,+2	1.44 (+/- 0.14)	0.82 (+/- 0.10)
A-21T,T-22A	-88,+2	1.19 (+/- 0.12)	0.64 (+/- 0.08)
A-24T	-48,+1	1.40 (+/- 0.13)	0.94 (+/- 0.12)
G-25T	"	0.87 (+/- 0.20)	0.58 (+/- 0.14)
G-26T	"	1.00 (+/- 0.20)	0.67 (+/- 0.14)
C-28T	"	1.13 (+/- 0.25)	0.76 (+/- 0.18)
G-30C	"	1.57 (+/- 0.13)	1.05 (+/- 0.12)
T-33A	"	0.45 (+/- 0.11)	0.30 (+/- 0.08)
G-34T	"	-0.93 (+/- 0.47)	-0.62 (+/- 0.32)
T-35C	"	-0.80 (+/- 0.29)	-0.54 (+/- 0.20)
T-36G	"	-2.34 (+/- 0.43)	-1.57 (+/- 0.31) ^e
C-37G	"	0.90 (+/- 0.16)	0.60 (+/- 0.12)
C-37A	"	0.73 (+/- 0.23)	0.49 (+/- 0.16)
C-39T	"	1.34 (+/- 0.17)	0.90 (+/- 0.13)
C-39G	"	1.15 (+/- 0.13)	0.77 (+/- 0.11)
C-40T	"	1.53 (+/- 0.15)	1.02 (+/- 0.13)
C-40G	"	1.19 (+/- 0.22)	0.80 (+/- 0.16)
T-42A	"	1.07 (+/- 0.17)	0.72 (+/- 0.13)
A-44T	"	1.43 (+/- 0.07)	0.96 (+/- 0.09)
T-47G	"	1.67 (+/- 0.10)	1.12 (+/- 0.11)
INSERTIONS AND DELETIONS			
Ains-22	-50,+2	0.37 (+/- 0.17)	0.21 (+/- 0.10)
Tins-23	-48,+1	0.53 (+/- 0.17)	0.35 (+/- 0.12)
Ains-25	-50,+2	0.39 (+/- 0.22)	0.22 (+/- 0.13)
Ains-25	-88,+2	0.61 (+/- 0.21)	0.33 (+/- 0.11)
Cins-29	-50,+2	0.46 (+/- 0.22)	0.26 (+/- 0.13)
Cins-29	-88,+2	0.25 (+/- 0.20)	0.13 (+/- 0.11)
Tdel-18	-48,+1	-0.39 (+/- 0.75)	-0.26 (+/- 0.50)
Adel-20	"	0.32 (+/- 0.57)	0.22 (+/- 0.38)
Cins-18,C-27T	"	0.83 (+/- 0.26)	0.55 (+/- 0.18)
C-17G,Cins-27	"	0.74 (+/- 0.15)	0.50 (+/- 0.11)

^a Sequence limits are given with respect to the expected transcription start site, although the actual start site was not determined for the promoter mutants.

^b The values of the slope and standard deviation were determined as described in the Materials and Methods section.

^c This ratio (fraction of regulation) and standard deviation were determined by using the scaled slope from the previous column divided by the wild-type (WT) slope with the same sequence limits, as described in the text.

^d The rac145 promoter (15) has a 4-bp substitution at positions -20 through -23. Its slope was compared with that of the [-88, +2] wild-type promoter.

^e At higher growth rates, the β -galactosidase activities in these cases were very close to background. Therefore, although the slopes are clearly negative, the absolute values are subject to large errors.

Like the G+C-rich sequence, parts of the spacer, the -10 and -35 hexamers, and the 16-bp spacing between the two hexamers are all highly conserved among the P1 promoters of the seven *E. coli* rRNA operons (27). Unlike the G+C-rich sequence, however, these other determinants and the spacer sequence are not as strongly conserved among tRNA promoters (27). From gene dosage experiments, it appears that tRNA operons are under the control of the same negative feedback system that regulates rRNA promoters (16, 17, 28, 39). Since their promoter sequences differ from those of the rRNAs, it could be that tRNAs are regulated slightly differently than rRNAs, consistent with the observation that there are small but reproducible differences between rRNA and tRNA promoters in the level of repression observed in the gene dosage experiments cited above. Additional experiments will be needed to address this question.

Mechanism of growth rate regulation. Promoter mutations leading to a loss of growth rate-dependent regulation in vivo potentially belong to one or more of the following classes. (i) The mutation could interfere with the interaction between RNA polymerase (RNAP) and the promoter so that the kinetic step originally affected by the regulator is no longer the major contributor to the overall rate. (ii) The mutation could directly alter an interaction between the promoter and a regulatory molecule (whatever its identity). (iii) The DNA segment containing the mutation could alter the conformation of the bound RNAP so that RNAP no longer interacts normally with the effector. The phenotypes of the mutant promoters suggest molecular mechanisms for growth rate regulation that can be tested in vitro. Measurement of the mutant promoters' kinetic parameters in vitro under conditions in which RNAP binding to rRNA promoters approaches physiologically relevant rates (14) could distinguish between different molecular models independent of the identity of the regulator.

The unregulated promoters can be grouped into three categories: those with reduced activities, those with increased activities, and those with activities relatively close to the wild-type level. Mutations leading to drastically reduced activities, i.e., those in the -10 and -35 consensus hexamers and those making 15-bp spacers, might fit in the class of changes that affect not the regulatory interaction, but the interaction of the promoter with RNA polymerase. That is, the mutation might slow down one kinetic step substantially, making the effect of the regulator on another step less important to the overall rate.

The loss of regulation in the mutants with increased activities (T-33A, insertions in the spacer) could result from any of several possibilities. As with the promoters with low activities, the high-activity, unregulated promoters could have altered interactions not with the regulator, but with RNA polymerase, i.e., these promoters could have altered rate constants which make the effects of the regulator inconsequential. A second possibility is that these promoters could interact better with RNAP, so that an effector-modified form of RNAP could no longer compete and therefore exert effects on transcription. A third explanation for the loss of regulation in these mutants could be that these promoters have lost the requirement for a positive activator (which is negatively regulated as growth rate decreases). Finally, a fourth explanation for the loss of regulation is that the target of a negative regulator (on the DNA or on the RNAP) has been altered; the promoter is derepressed.

The mutations causing defects in regulation which are most likely to involve altered interactions with a regulatory

molecule are those in positions not normally considered as participating in direct promoter-RNAP interactions, e.g., [C-1T,C-15G] and [C-19A,A-20C]. [C-19A,A-20C] showed activity close to the wild-type level but had radically altered regulation. The phenotype of this mutant might be a composite of two effects: a promoter that is intrinsically less active and that is unable to interact with a regulator and is therefore derepressed. Alternatively, the mutations might not change RNAP-regulator interactions directly, but could change regulation by inducing changes in RNAP that prevent RNAP-effector interactions, as recently proposed for lambda p_{RM} -RNAP-repressor interactions (21). Two other mutants also implicate this region of the spacer in regulation. A 4-bp change at -20 to -23 (rac145) and two single substitutions (C-19T and A-20G) had an altered growth rate response.

Effects of proposed regulators in vitro. The experiments discussed here do not address the identity of the negative regulator of stable RNA promoters or the mechanism by which that regulation is accomplished. Various models have been suggested (reviewed in reference 34). A strict inverse correlation between the rate of rRNA synthesis and ppGpp concentration has been well established (3, 17, 36, 38), which has led to the proposal that ppGpp is a mediator of both stringent and growth rate control by inhibiting in some way the productive interaction between rRNA promoters and RNA polymerase. The determinants of growth rate control described here clearly include the consensus sequences involved in recognition by RNA polymerase and sequences adjacent to the consensus hexamers. Therefore, models in which a regulator (ppGpp and/or another factor) causes changes in the DNA recognition properties of RNA polymerase are consistent with these results. However, other models in which a regulator competes with RNA polymerase for overlapping DNA sequences are clearly not excluded.

A common model for rRNA regulation is that there is a ppGpp-dependent partitioning of RNAP into two conformations with different promoter selectivities (3, 45). Either the ppGpp-modified form, which cannot recognize stable RNA promoters, becomes predominant in the RNAP population, making the unmodified form limiting, or the modified form actively interferes with and inhibits transcription at stable RNA promoters. We have argued that if this model is true, then the ribosome concentration (free or translating) in some way determines the ppGpp synthesis rate, by monitoring the protein-synthetic capacity relative to the nutritional state of the cell (34).

If ppGpp is sufficient to enact the partitioning of RNAP into different forms, then there might be derepressed mutant promoters (increased activity in vivo) which either are transcribed normally by or are insensitive to the ppGpp-modified form. These might have wild-type activities in vitro and be unresponsive to the presence of ppGpp, while a wild-type promoter would be inhibited by ppGpp in vitro. In contrast, mutants with increased regulation might be more sensitive to the presence of the effector in vitro.

Stringent control. We have not yet tested the mutant promoter constructs for stringent control in vivo. It is likely, although unproven, that both growth rate-dependent and stringent control employ the same mechanism for control of stable RNA synthesis (3, 38). Therefore, we would expect that mutants identified here as unregulated would also be defective in stringent control.

Role of -10 and -35 spacer region. As noted previously (2a, 8, 19, 20, 33), 17-bp spacing between the -10 and -35 regions is the consensus for *E. coli* promoters, and mutants

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